Interaction of fear and stress: from healthy population samples to post-traumatic stress disorder

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June 6th 2011

A thesis submitted to McGill University as partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

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Acknowledgements

I began my PhD in September 2005 after having completed a Master's degree in the department of Psychiatry. In the past years, many people have directly or indirectly contributed to this body of work, this *opus* that follows. It may sound like a cliché, but it would not have been possible without the following. So, to you all, I humbly thank you.

To my supervisor, Dr Jens Pruessner, I thank you for your supervision, expertise, trust in me and willingness to embark on such an ambitious project. I can still remember our conversation in New Orleans, when you asked me what the difference between stress, fear and anxiety was. Little did I know at the time that it would the question that would haunt me and guide my research for the following years. These projects have taken me on a rollercoaster of highs and lows. But through all the uncertainty, questions, doubts, celebrations and stressful times, you've reminded me of what it takes to become a good scientist. Some projects work out, others do not. But you've taught me to never forget the basic reason that motivates our work: science is simply amazing.

To my advisors, Dr Karim Nader and Dr Alain Dagher, I thank you for the support and advice you've provided me since the beginning. Despite changes in plans, you have provided me with critical perspective and support. You have always asked me the right question at the right moment. I thank you both for this.

To Dr Alain Brunet, I thank you for allowing me to begin my graduate studies. Your expertise in PTSD has been a great guidance and source of many questions that I will hopefully continue to explore in the coming years. Thank you for providing me access to the PTSD data presented in this thesis.

The projects presented in this thesis could not have been done without the help of specific people that deserve my humble thanks. To Lindsey Moncarz, thank you for your time and dedication. Your help for recruitment of participants, phone screening, prepping material and entering data has allowed this project to work out. Your smile and work ethics will not be forgotten. To Justin Singh, your help in testing participant and entering data will not be forgotten. Your ever-positive attitude makes working with you a real pleasure. À Yann Quidé, un énorme merci pour m'avoir accompagné dans les analyses du cortisol. Ton séjour parmi nous fut bref, mais c'est toujours un plaisir de te recroiser. To Thomas Beaudry, our computer research assistant, thank you for teaching me (with stoic patience) the fun of programming and providing me with technical help for the GSR analyses and cortical thickness software. These projects would have taken me a decade more if it wasn't for your work. To Catherine D'aguiar, our research assistant, thank you for helping manage the paperwork. It is sometimes a daunting task to see through the forms to be filled, completed, signed, photocopied seventeen times and stamped, reapproved and co-signed. Your help will not be forgotten. You are a great friend and colleague.

To all the volunteers that helped in the actual testing, our own panelists that would be there anytime with such enthusiasm to help out, a very humble thanks. Devin Sodum, Natalie Gottlieb, Armen Bazarian, Sandra Pietrantonio, Stephanie Dimitroff, Ellen Stephenson, I'm grateful for having worked with you. To Maria Ioannou, fellow IPN student and friend, thank you for the great editing work you have done. Your questions and comments have helped me enormously when I needed it most. To my fellow Douglas colleagues, thank you for making work such a great place to be. Special thanks go to Ian Hellstrom and Lindsay Naef, without whom the student committee would not be what it is. It's been a pleasure working with you to make student life at Douglas better. No matter where our roads take us, we will meet again, probably for drinks!

My dear Julie Andrews, friend, colleague and roommate, thank you for being you. You've been there to help, counsel, give perspective, edit what I wrote, question my stats, and remind me why I do what I do. You've been a steady friend, one that I am ever grateful to have in my life. It's been a long ride, but you made it fun. From the bottom of my heart, thank you.

My dear Nida Ali, friend and colleague, thank you for being you. You are a fierce friend and an awesome colleague. Never would I have known that such a small package could contain such a great person. It's been a great time having you sitting in front of me. Your help in editing and your moral support helped me get through some tough spots. I won't forget it.

Ma chère Catherine Lord, Catou, un énorme merci du fond du coeur. Tu as été une collègue et mentor incroyable. Ton support, du début à la fin, a été une bénédiction. Un gros merci de m'avoir rappelé que la vie ne s'arrête pas après le doctorat. Merci pour les mille conversations, les conseils, la perspective, et un indéfectible sourire qui sait remonter le moral quand il est à plat. Tout simplement, merci.

To my lab mates, Simona Efanov, Katarina Dedovic, Annie Duchesne, Cory Cooperman, Veronika Engert, it's been a long way home. But you have all provided me with help and support, and for that, I thank you humbly. Wherever our roads may take us, it will always be a privilege to see you.

Je voudrais adresser mes plus sincères remerciements à ma famille. À mon père et ma mère, un énorme merci pour votre soutient, votre patience, votre compréhension. Vous avez toujours su me rappeler que je devais suivre mes rêves, aussi fous soient-ils. Vous m'avez offert tout ce dont un fils pourrait avoir besoin et plus. Si je suis rendu où j'en suis, c'est grâce à vous. À mon frère et ma belle-soeur, je vous offre un énorme merci. Vous avez su au cours des années m'offrir de judicieux conseils, un support unique et des moments où le stress et l'angoisse n'existaient plus. Ces moments, si courts soient-ils, ont su me rappeler de profiter de la vie hors du travail afin de mieux travailler. Cet équilibre, vous m'avez aidé à le maintenir. Merci du fond du coeur. Et un gros merci à mon neveu. Merci d'être ce petit rayon de soleil qui sait me faire rire peu importe le stress.

Un gros merci à mes amis, Nick, Mélody, Isabeau et tous les autres, de m'avoir accompagné dans ce projet. Merci d'avoir été patient quand je n'avais pas le temps de vous voir. Merci de m'avoir gardé en contact avec le monde extérieur! Une touche de réalité fait toujours du bien.

It's been an intense six years. I was never alone in this, so to all those who walked with me, thank you. Your words, advice, help and smiles will never be forgotten or taken for granted. Thank you.

Contributions of Authors

The following thesis contains four chapters that cover distinct studies. These studies will be edited to become manuscripts to be submitted to peer-reviewed journals. The following section reviews the individual contributions to each project for every co-author.

Chapter 2

Corbo, V.: literature review, study design, task development, recruitment and testing of subjects, GSR and endocrine data analyses, data entry, manuscript writing and editing (90%).
Beaudry, T.: computer assistance in task development
Pruessner, J.C.: project supervision, study design consultant, manuscript editing

Chapter 3

Corbo, V.: literature review, study design, task development, recruitment and testing of subjects, GSR, heart rate and endocrine data analyses, data entry, manuscript writing and editing (90%).
Beaudry T.: computer assistance in task development
Moncarz, L.: subject recruitment and screening, data entry
Pruessner, J.C.: project supervision, study design consultant, manuscript editing

Chapter 4

Corbo, V.: literature review, testing of subjects, endocrine analyses, data entry, manuscript writing and editing (80%)

Quidé, Y.: endocrine analyses, data entry

Brunet, A.: study design, project supervision, manuscript editing

Pruessner, J.C.: study design, project supervision, manuscript editing

Chapter 5

Corbo, V.: literature review, testing of subjects, cortical thickness analyses, data entry, manuscript writing and editing (80%)

Beaudry, T.: software design for cortical thickness analyses

Brunet, A.: study design, project supervision, manuscript editing

Pruessner, J.C.: study design, software design consultant, project supervision, manuscript editing.

ABSTRACT

Fear and stress are two closely related psychological concepts. At the biological level, activity of the sympathetic nervous system (SNS) measured through galvanic skin response (GSR) is considered as a marker of fear in humans. In parallel, the secretion of cortisol consequent to the activation of the hypothalamic-pituitary-adrenal (HPA) axis has been identified as a reliable marker of stress. However, few human studies have investigated the interaction of endogenous cortisol and GSR in a pavlovian fear-conditioning design. Further, fear-conditioning has been used as a model for Post-Traumatic Stress Disorder (PTSD). This disorder is thought to be a failure to suppress exaggerated fearful reactions acquired at the time of trauma. Cortisol, as the main stress hormone, has been hypothesized as a potential modulator of the fearful reactions observed in PTSD. However, it remains unclear if PTSD is mostly a fear-based disorder or if symptoms may be associated to other factors, such as cortisol and brain structures, that are not part of the fear network.

The work presented in this thesis followed two parallel lines. The two first chapters investigated the interaction between cortisol and GSR reactivity in healthy volunteers. We demonstrated that exposing subjects to a fear-conditioning paradigm was not enough to induce a cortisol response. Further, we observed a greater reactivity in women. In our second study, our results showed that an endogenous cortisol rise induced prior to extinction was associated with a faster decrease of the GSR response to the conditioned stimulus. Replicating our first study, we found that women reacted more to the conditioning paradigm compared to men. Lastly, while cortisol secretion was correlated with

childhood adversity and anxiety trait, GSR reactivity did not correlate with personality measures.

Our second line of investigation targeted civilians exposed to trauma. In our third study, we observed that increased levels of cortisol in response to awakening were associated with resilience to trauma. Furthermore, based on previous work investigating central nervous regulators of the HPA-axis and fear reactivity, our investigation of cortical thickness of individuals recently exposed to trauma confirmed the expected thinner ACC. We also highlighted the association between ventral temporal cortex and frontal pole with symptoms severity. These regions add a cognitive and social dimension to PTSD severity that may share more with stress than fear itself. These two studies argued for a more comprehensive model of PTSD that includes both fear-conditioning and stress reactivity to better account for the wide scope of symptoms.

I conclude this thesis by re-examining the current proposed model for interaction between cortisol and peripheral measures of fear. I review the influence of sex as a mediator of fear acquisition, reactivity to stress and extinction of fear. Finally, I extend these findings to our PTSD studies to evaluate the use of pure fear-conditioning as a model for PTSD symptoms emergence and maintenance.

RÉSUMÉ

La peur et le stress sont deux concepts psychologiques intimement reliés. Au niveau biologique, l'activité du système nerveux sympathique (SNS), mesuré par la réponse électrodermale (RÉD), est considéré comme un marqueur de la peur chez l'être humain. Parallèlement, la sécrétion de cortisol suite à l'activation de l'axe hypothalamo-hypophyso-adrénergique (HHA) est le marqueur le plus commun du stress. Cependant, peu d'études se sont penchées sur l'interaction entre le cortisol et la RÉD lors d'un conditionnement de peur pavlovien chez l'être humain. De plus, le conditionnement de peur est utilisé comme modèle pour étudier le Trouble de Stress Post-Traumatique (TSPT). Ce trouble est considéré comme un échec de supprimer une réaction de peur exagérée acquise lors du traumatisme. Le cortisol, en tant qu'hormone de stress principale, est considéré comme un agent qui influencerait la force des réactions de peur dans le TSPT. Cependant, il demeure incertain si le TSPT est principalement un trouble relié à la peur ou si sa symptomatologie est relié à d'autres facteurs, tels le cortisol ou des structures neurologiques qui ne sont pas associées au système de la peur.

Les travaux de cette thèse suivent deux lignes parallèles. Les deux premiers chapitres présentent les résultats de l'étude de l'interaction entre la peur et le stress chez des participants en santé. Nous illustrons que l'exposition à un conditionnement de peur n'est pas suffisant pour provoquer une réponse de cortisol. De plus, nous avons observé une plus forte réactivité au conditionnement chez les femmes. Les résultats de notre deuxième étude indiquent qu'une augmentation de cortisol endogène est associé à un déclin plus rapide de la réponse

au stimulus conditionné lors de l'extinction. Cette étude confirme aussi une plus forte réactivité chez les femmes. Enfin, alors que la sécrétion de cortisol est associée à l'adversité durant l'enfance et l'anxiété, la RÉD n'était pas associée aux traits de personnalité.

Parallèlement à ces études, nous avons étudiés des civils exposés à un événement traumatique. Notre troisième étude montre qu'une réponse accrue de cortisol en réaction au réveil est associée à la résilience face à un événement traumatique. De plus, notre étude de l'épaisseur corticale a confirmé que, chez des individus récemment exposés à un événement traumatique, le cortex cingulaire antérieur est correlé négativement à la sévérité des symptômes. Cette étude a aussi mis en lumière deux nouvelles structures, le cortex ventro-temporal et le pôle frontal, qui sont associées à la sévérité des symptômes. Ces deux structures ajoutent une dimension cognitive et sociale à la sévérité du TSPT et sont associés plus fortement au stress qu'à la peur en soi. Elles suggèrent donc un modèle d'étude qui va au-delà du conditionnement de peur et qui intègre l'importance du stress pour mieux décrire la symptômatologie.

Je conclue cette thèse en réexaminant le modèle d'interaction entre le stress et les mesures périphériques de la peur. Suivant cela, j'examine le sexe comme médiateur possible dans l'apprentissage de peur, la réactivité au stress et l'extinction de la peur. Enfin, je fais le pont entre les premières études et celles sur le TSPT pour évaluer l'usage du pur conditionnement de peur comme modèle pour décrire l'émergence et le maintient des symptômes.

Chapter 1: INTRODUCTION

1. FEAR

1.1 What is Fear?

The author Howard Philips Lovecraft (1890-1937) once said: “Fear is the oldest and strongest emotion of Mankind”. This saying eloquently reveals two important aspects of this common emotion, for which the vernacular vocabulary possesses a rich and colorful list of synonyms, such as fright, terror, horror, and panic, for example. If asked to describe what fear is, most lay people would reveal interesting psychological and physiological components that accompany the subjective experience. “Palm sweating”, “heart racing”, and “shortness of breath” would probably come as no surprise. What this illustrates is that, although fear may appear as a subjective emotion, it is fundamental to the human experience. Some events of the past years have showed us that fear triggers a set of reactions that can command full control of individuals, independent of ethnicity, culture, age, gender or creed. Fear can be a useful tool in certain situations, an adaptive set of behavior that increases the chances of survival of an individual faced with a significant threat to its life or integrity. However, in some cases, fear can become overpowering, extending beyond its adaptive mandate and becoming a crippling disorder, as is the case of Post-Traumatic Stress Disorder (PTSD, see below).

The typical responses associated with fear are strikingly similar across species and fall into three distinct patterns: flight, fight and freeze. These responses are apparent in the natural context as well as

in the laboratory. They offer quick and efficient ways to deal with an immediate threat to survival. In nature, for example, rodents will display fearful behavior when present in open spaces, where they are exposed to threat and potential predators. Similarly, humans display common fearful reactions to electrical shocks. The question that has been the object of many studies has focused on how individuals learn what to fear and what not to fear. Many stimuli are often presented at the same time as the actual threat. Thus, the individual may learn to react to the stimuli associated with that threat. In order to better understand this, researchers moved towards the laboratory, modeling fear as an associative learning based on the works of Ivan Pavlov.

1.2 Fear-Conditioning Model

1.2.1 Acquisition

The first question one must ask is how individuals, whether rodents or humans, become afraid of things. Pavlovian fear-conditioning studies have illustrated how a neutral stimulus, if paired in a temporal manner to a naturally aversive stimulus (unconditioned stimulus, US), can evoke the fearful behavior (conditioned response CR), even in the absence of the US. Thus the previously neutral stimulus becomes the conditioned stimulus, (CS+), contrasted with a neutral stimulus that has never been paired with the US (CS-). This paradigm has been used to study fear across species, from rodents such as mice or rats to primates and humans (Delgado, Olsson, & Phelps, 2006). It is crucial to mention that the learning is not purely

based on a simple temporal relationship between two stimuli, but rather on the fact that the organism gains information about the causal relationship between stimuli (Rescorla, 1988b). Specifically, the CS+ becomes seen as a causal predictor of the US. Organisms do not simply associate, but attempt to understand laws governing their world.

One important element to note about fear-conditioning studies is the nature of the stimuli used as the CS and US. In nature, the threats are obvious and often related to what would constitute a CS (Domjan, 2005; Ohman & Mineka, 2001). For example, the shadow of a bird of prey can induce fear without possessing inherent threatening properties. However, the shadow is related to the bird by shape and movement. The same could be said of the sound of hissing, as it is related to the snake that emits it. In the laboratory setting, the conditions present in nature cannot be fully reproduced. However, studies have shown the possibility to induce fearful learning through the use of artificial stimuli. Examples of CS used in studies on human subjects include items ranging from colored lights [e.g. (Barrett & Armony, 2006; Bechara, et al., 1995; Cheng, Knight, Smith, Stein, & Helmstetter, 2003; Grillon & Ameli, 2001; Grillon, Cordova, Morgan, Charney, & Davis, 2004; Jovanovic, et al., 2006; Lang, et al., 2009)], geometrical figures [e.g. Brignell & Curran, 2006; Knight, Smith, Cheng, Stein, & Helmstetter, 2004; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Norrholm, et al.; Otto, et al., 2007; Tabbert, et al., 2010; Vervliet, Vansteenwegen, Baeyens, Hermans, & Eelen, 2005], pictures of dangerous animals like spiders and snakes [e.g. Courtney, Dawson,

Schell, Iyer, & Parsons, 2010; Zorawski, Blanding, Kuhn, & LaBar, 2006; Zorawski, Cook, Kuhn, & LaBar, 2005], pictures of faces [e.g. Birbaumer, et al., 2005; Jackson, Payne, Nadel, & Jacobs, 2006; Morris, Ohman, & Dolan, 1998] and even Rorschach and gradients [e.g. Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Moratti, Keil, & Miller, 2006; Schultz & Helmstetter, 2010]. The point here is not to review every study published using this paradigm (for a review, see Pineles, Orr, & Orr, 2009) but to illustrate that most studies use visual stimuli of varying complexity, from very easily processed ones like simple colors to more complex ones like gradients. Also, with the improvement in technology, some studies have even begun to use virtual reality in order to increase the realism of the context with landscapes (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; M. R. Milad, et al., 2005). The level of complexity of the stimuli may in themselves influence the general level of arousal in the task: more realistic, complex, novel or threatening stimuli could be viewed as more arousing relative to simple geometrical figures and colors. This arousal may increase the possibility of the CS-US association (Ohman & Mineka, 2001) but it may also distract the participant from the association, especially if the US is not salient or aversive enough. In a review of conditioning as a learning method, Domjan (Domjan, 2005) has argued a functional perspective, illustrating that some stimuli might serve better as CS if they possess a functional significance for the subjects. This line of reasoning is based on the theory of bio-preparedness (Ohman & Mineka, 2001), that underlies how specific stimuli possess an inherent capacity to be associated faster to US in a pavlovian conditioning paradigm. For example, pictures of snakes would allow for

stronger association with a US compared to triangles, because of the innate threat of snakes that has been conserved through evolution. Some stimuli would therefore possess a greater capacity of predicting the occurrence of an aversive stimulus. Purely artificial stimuli like simple geometrical figures would not be very good predictors, whereas pictures of animals like snakes and spiders, that are by nature threatening, would make the association with the US easier.

In the laboratory setting various US have been used to induce conditioning. While most studies cited above used a mildly painful electrical shock applied on the shins or wrists, some studies have used other US such as a burst of loud noise (Barrett & Armony, 2006; Bechara, et al., 1995; Courtney, et al., 2010; Guimaraes, Hellewell, Hensman, Wang, & Deakin, 1991; Hoefer, et al., 2008; Moratti, et al., 2006; Morris, et al., 1998), puffs of air blasts (Jovanovic, et al., 2006; Norrholm, et al., 2010) or even odors of rotten eggs (Gottfried & Dolan, 2004). The first issue here is that all these stimuli target different sensory systems, some which may have greater access to the neurological fear network. Another limitation to the exact comparison is the relative unpleasantness of the stimuli: electrical shocks induce pain, whereas noises will likely be startling but not painful. Noxious odors may simply be unpleasant and share more with the emotion of disgust than they do with fear itself. This raises the issue of where fear ends and where pain begins: in nature, a prey would fear a predator, but once the attack has landed, fear would become mixed with pain. Interestingly, some studies have highlighted that the fearful anticipation and actual experience of pain are linked to the

same brain structures. Specifically, the anterior cingulate cortex, to which we will return below, is activated by both anticipation and experience of pain (Ploghaus, et al., 1999; Porro, et al., 2002; Vogt, 2005). This mix of sensations and brain structures might be why mildly painful electrical shocks are most often used, as they are more aversive through the combination of these sensations.

Beyond the nature of the stimuli used, other factors may influence the rate and strength of acquisition. One is the contingency of pairings between CS and US. Experimental designs must present enough US for the CS to be perceived as threatening while not presenting the US so often that subjects habituate to its presence. Rescorla (Rescorla, 1988a) considers this parameter to be paramount in the efficiency of a conditioning paradigm. His model describes how the contingency conveys the essential causal information between stimuli. Therefore, conditioning depends on the ability of the CS+ to predict the presence *as well* as the absence of the US. This highlights the care one must take in selecting presentations of unpaired CS+ as well as unpaired US during training. Another element other authors have reported which must be taken into account is the temporal association between CS and US. In some studies, the CS and US occur simultaneously, a design termed trace conditioning, whereas other studies present a gap between the two stimuli, i.e. delay conditioning. This temporal gap increases the demands on the organism to associate the two stimuli and may result in a weaker learned association than with trace conditioning. This may be due to demands on structures in the central nervous system or, closer to what Rescorla argued,

because there is a natural temporal relationship between various stimuli (e.g. a taste aversion using a nauseous reaction as US should present a temporal gap between ingestion of food and the bodily reactions that mimics best a natural digestion, whereas the temporal gap between a visual stimulus and electricity-induced pain should be relatively quick). Also related to the temporal dimension, studies have used various inter-trials intervals (ITI) in their designs. ITIs refer to the time lapse between training with some studies maintaining constant ITI, and others randomizing the length. The length of time between stimuli may be interpreted as a predictor of the US and may come to rival the CS itself in eliciting the fearful response.

In sum, pavlovian fear-conditioning is an excellent paradigm used to replicate the processes of learning fear in the laboratory. Its efficiency is demonstrated and also limited by the fact that even artificial stimuli can elicit fearful reactions. One important question that remains to be studied is; once people have learned to fear a stimulus, how can they learn to stop?

1.2.2 Extinction

The process responsible for the decline of a fearful response after successful acquisition of fear conditioning has been termed “extinction”. When subjects are presented with the CS+ without any occurrence of the US, over time, the CS loses its capacity to elicit the CR. This can be seen in the decrease percentage freezing in rodents exposed to unpaired CS+ (Quirk, et al., 2010). This process does not

require many trials, as animals and humans are generally able to discriminate when not to respond. One important question that has been extensively investigated in rodents has focused on the nature of extinction. Specifically, studies have investigated if extinction is an erasure of the CS-US association or if, as proposed by Pavlov himself, it is a new learning that inhibits the expression of the CR (Bouton, 2004). Evidence using two different procedures seem to indicate that the association between the CS and US is not lost, but that extinction is a new learning that super-imposes itself on the original learning. First, a procedure called "renewal" shows how simply changing the context can provoke the reappearance of the CR after extinction learning. In other words, if extinction is learned in a specific cage, moving the rat to a different cage and presenting the CS can trigger the CR as strongly as it was prior to extinction learning. This highlights the importance of the context in extinction learning. A second line of evidence comes from "reinstatement" paradigms, in which an unpaired US is delivered to the rat, triggering the reappearance of the CR to a CS+. Functionally, this means that while the individual can learn to suppress a reaction, the initial fearful reaction is still present in the behavioral repertoire, in case a real threat is present and the individual needs to respond. Reacting to a false positive is more advantageous than ignoring a real threat, despite the loss of energy.

1.3 Quantifying the Fear Response

1.3.1 Galvanic Skin Response

In order to assess the efficiency of stimulus learning, a natural response must be observed and quantified. This response has traditionally been quantified for rodents as the amount of time they exhibit freezing behavior to the CS+ relative to the CS- (Cahill, Pham, & Setlow, 2000; J. E. LeDoux, 2000; Debiec & Ledoux, 2004; Barnes & Good, 2005; Bucherelli, Baldi, Mariottini, Passani, & Blandina, 2006; Cai, Blundell, Han, Greene, & Powell, 2006; Debiec, Doyère, Nader, & LeDoux, 2006; Delgado, et al., 2006; Chang & Maren, 2009; Duvarci, ben Mamou, & Nader, 2006). Human studies have relied on other measures to quantify the amount of fear displayed by participants, such as the galvanic skin response (GSR). This response represents the changes in the natural occurring electrical resistance of the human glabrous skin. During an emotional experience, norepinephrinergic projections from the central nervous system activate the sympathetic component of the peripheral nervous system. This results in modifications of the thermoregulatory input to the eccrine sweat glands present across the skin (Critchley, 2002). This response is part of a pattern of autonomic and motor responses serving the flight-fight-freeze repertoire. It is also related to emotional reactions through an increase of arousal (Kreibig, 2010). Therefore, emotional experiences modify the natural electrical resistance of the skin by controlling sudation; the greater the arousal that follows an emotional experience, the greater the activation of the sympathetic nervous system (SNS) and hence, the

greater the change in GSR due to increased opening of the sweat pores. This method has the advantage of being a non-invasive way to quantify real-time emotional responses. However, since activations of the sympathetic nervous system are not emotion-specific, i.e. that the SNS will be activated in a similar degree by various emotions, GSR cannot be considered a marker of fear *per se*. This raises the issue of the exact stimuli used to induce the emotion, to which we will come back to later.

1.3.1 Technical Considerations in Fear-Conditioning

Despite a great deal of interest on studies of fear conditioning, a lot of confusion stems from understanding the fear-conditioning paradigm itself. Fear-conditioning posits that you can create a CR through the association of any two stimuli, independent of their exact nature, as long as one neutral stimulus becomes predictive of a noxious one. We have already illustrated the wide variety of stimuli used to induce conditioning, as well as the possible timing of the stimuli (trace versus delay conditioning, ITI). Beyond the nature of the stimuli, we must mention the actual mechanics of the acquisition phase. First, not all studies expose their participants to the same amount of stimuli throughout the task: some have shown effects with as little as 8 stimuli in total (Jackson, et al., 2006; Vervliet, et al., 2005), while other studies use up to and over 30 stimuli (Gottfried & Dolan, 2004; Moratti & Keil, 2005; Schultz & Helmstetter, 2010; Zorawski, et al., 2006; Zorawski, et al., 2005). The next variable to include is the contingency of pairings between CS and US: most studies reviewed

here apply a 100% rate of pairing during acquisition, though some have gone as low as 50% or even 33% (Phelps, Delgado, Nearing, & LeDoux, 2004; Zorawski, et al., 2006; Zorawski, et al., 2005). The exact number of pairings needed is something that has yet to be determined, as one runs the risk of creating habituation if the exposure is done too often. On the other hand, too few exposures may not create enough association simply because it was not repeated often enough for the CS-US relationship to be learned. The optimal parameters have not been extensively studied and leave the author at liberty to find an optimal workable paradigm.

Further, we must mention that the actual data reported varies from one laboratory to another. This may be due to how the actual data reported is transformed from the raw values. Most studies report the data transformed with either a natural logarithm (ln+1) of the data or a square root (SQRT) transformation. Both these transformations are meant to normalize the data, as GSR is an extremely variable measure between individuals. It must be highlighted that, while both transformations are an acceptable way of transforming data to increase normality, one cannot be directly compared to the other. For example, if the contrast between peak and baseline yields a value of 0.45 microSiemens (μS), the ln+1 transformation would result in a value of 0.3716, whereas the SQRT would yield a net value of 0.6708. We must further note that, while it is possible to use the SQRT of absolute values for negative responses, it is impossible to use the same methods for the ln+1 transform, as the curve of the logarithmic function increases exponentially for

values that are between 0 and 1. Therefore, all negative responses in GSR must be excluded from analyses if one follows the ln+1 transformation.

While these technical issues may limit our capacity to directly compare the numbers obtained in various studies, it remains that fear-conditioning as a theoretical model has been successfully used in many studies across species to help us understand the biological, and more specifically, the neurological basis of such learning. The next section reviews these studies, based on the neuroanatomical structures that contribute the most to the various steps of fear acquisition as well as extinction of fear.

1.4 Neurobiology of Fear-Conditioning

1.4.1 Amygdala

Early neurological studies have identified the amygdala as the core neural center responsible for acquisition and expression of fear-conditioning. The amygdala is a small set of nuclei located bilaterally in the anterior portion of the medial temporal lobes. It is a phylogenetically ancient structure present in the mammalian brain across species (J. E. LeDoux, 2000). It sits anterior to the hippocampus; another important structure in fear conditioning that will be described below. It receives afferents directly from the sensory areas of the thalamus that bypass the main sensory areas, such as the visual cortex. It also receives projections from the anterior

cingulate cortex and ventro-medial prefrontal cortex. In return, it projects to the motor areas of the thalamus and to the hypothalamus. This is the pathway that triggers the activation of the SNS.

The implication of the amygdala in fear-conditioning in rodents has been extensively reviewed (J. LeDoux, 2003). The interesting aspect is that those results have been replicated in humans as well. In an early review of the field, McGaugh and colleagues demonstrated how the amygdala is essential for creating and enhancing new emotional memories, both in rodents and in humans (McGaugh, Cahill, & Roozendaal, 1996). More recently, with the advancement of brain imaging techniques, new data has emerged further highlighting the importance of this small complex in the formation of fear conditioning and emotional memory (Cahill, et al., 1996). One crucial study by Bechara and colleagues (Bechara, et al., 1995) examined patients with focal lesions to the amygdala and illustrated that this structure was essential for the associative learning of CS-US. They showed that patients with amygdala lesions would react to the US but would not be able to form the association with the CS, and hence never displayed increased CR to the CS+. Another landmark study by LaBar and colleagues (LaBar, et al., 1998) investigated the activation of the amygdala during a fear-conditioning task in healthy volunteers, both at the acquisition and extinction stages. Their results indicated that the right amygdala saw a 0.86 increase in signal in response to the CS+, compared to a 0.06 increase to the CS-. This illustrates the importance of the amygdala in learning specifically fear. Interestingly, the data further reported that the increase in spatial extent of the

activation observed in the amygdala during acquisition correlated significantly with the GSR exhibited by the same subjects. This supports the use of GSR as a reliable index of fear acquisition and central nervous system activation. This implication of the amygdala and concurrent GSR as a marker of conditioning was replicated by Morris and colleagues (Morris, Buchel, & Dolan, 2001) in a design where subjects were unaware of the contingency of pairing between the CS and US. Specifically, these authors used a backward masked angry face as CS lasting 30ms, followed by a neutral face lasting 45ms. Subjects generally reported that they were unaware of the masked face despite the increase in amygdala activation. This illustrates the possibility to induce conditioning without awareness or explicit knowledge of the contingency. Furthermore, their data indicated once more that the activity of the amygdala correlated with the GSR.

These studies, both in rodents and humans, highlight the importance of the amygdala for the creation of the CS-US association. The amygdala is also responsible for the expression of the CR through dense connections to the posterior hypothalamus and to motor centers, allowing for the triggering of the flight, fight or freeze response (Rodrigues, LeDoux, & Sapolsky, 2009). However, as crucial as it is, the amygdala does not work alone to create, support or eliminate fear. Oftentimes, elements unrelated to the specific CS-US association may modulate the expression of the CR. This leads us to the second neurological structure involved in fear-conditioning: the hippocampus.

1.4.2 Hippocampus

The hippocampus is another small structure located posterior to the amygdala, nestled in the medial temporal lobe. It extends from the posterior horn of the lateral ventricle to the amygdala. It receives afferents from the various sensory cortices through the input of the entorhinal, perirhinal, and parahippocampal regions, the amygdala and frontal regions. In return, the hippocampus projects to the frontal lobes, the amygdala and to the hypothalamus, amongst other regions. The hippocampus is a critical structure, being involved in various functions related mostly to learning and memory. A simple search for "hippocampus + learning + memory" on PubMed Central yields over a thousand review articles for this topic. It has been associated with episodic memory (Tulving & Markowitsch, 1998), verbal memory (Bonelli, et al., 2010), spatial orientation (Bohbot, Iaria, & Petrides, 2004; Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007), mood regulation (J. D. Bremner, et al., 2000), and stress regulation (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004; Dedovic, et al., 2010; Quirin, Pruessner, & Kuhl, 2008). In the context of fear-conditioning, the hippocampus' role is thought by some authors to be related mostly to contextual information encoding and storage in the dorsal portion of the hippocampus (Anagnostaras, Gale, & Fanselow, 2001; Fanselow, 2000; McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998). However, some authors still see a role in pure pavlovian conditioning (Bast, Zhang, & Feldon, 2001; Sanders, Wiltgen, & Fanselow, 2003), specifically through projections of the ventral hippocampus to the amygdala. In the case of fear, the concept of

context describes basically a set of spatial and temporal cues that, while not being directly connected to the US, may increase the probability of its occurrence. For example, a threatening mugger is always a danger, but the probability to be mugged increases in a remote location (alleyway versus a well-lit street) and at a specific time (middle of the night versus broad daylight). One study (McEchron, et al., 1998) has shown, that hippocampectomy in rats would prevent them from acquiring significant contextual fear-conditioning. This role of the hippocampus was also observed in humans in a context-based conditioning task conducted in the brain-imaging environment. In this experiment where the CS is determined not only by a discrete cue but also by the general landscape projected on the screen, subjects showed greater activity in the hippocampus to the CS+ compared to the CS- (Lang, et al., 2009). However, the main function that has been ascribed to the hippocampus is not at the acquisition stage, but rather at the extinction phase. It does this by providing cues about context that allow an individual to recognize elements that indicate safety versus danger (Bouton, 2004).

As mentioned in the previous section, extinction is not thought to be an erasure of the original CS-US association, but rather learning a new CS-no US association thereby inhibiting the CR. Contextual elements are thought to be responsible for the emergence of this new learning. This role of the hippocampus in extinction has been extensively investigated in rodents (Quirk & Mueller, 2008). Interestingly, it has been replicated in human studies using brain-imaging. In one study, Milad and colleagues (M.R. Milad, et al., 2007)

observed that subjects exposed 24 hours after initial conditioning and extinction displayed greater hippocampal activation to the stimulus that had undergone extinction compared to a stimulus that had been conditioned but not extinguished. Furthermore, the activity of the right hippocampus was positively correlated with decrease in GSR to the stimulus that had undergone extinction. The authors interpreted this finding in accordance with animal models that argue the role of the hippocampus in recall of extinction learning by actively inhibiting the amygdala's response to the CS+.

The data so far seems to implicate the hippocampus in both acquisition and extinction of fear-conditioning by analyzing contextual information and modulating the strength of the response to the CS+ (Ji & Maren, 2007). Another structure, the ventromedial Prefrontal Cortex (vmPFC), appears to be working in conjunction with the hippocampus to provide the decrease in response observed in extinction. We will turn to this structure in the following section.

1.4.3 Ventromedial Prefrontal Cortex

The vmPFC is located in the medial portion of the prefrontal cortex and encompasses structures such as the anterior cingulate cortex (ACC) and the orbito-frontal cortex. It is thought to serve many functions related to the interface between the cognitive and emotional centers of the brain (G. Bush, Luu, & Posner, 2000). For example, one study (Whalen, et al., 1998) used a modified version of the classic Stroop task to induce a conflict between emotion and cognition. In their

Emotional-Counting Stroop, the authors asked healthy subjects undergoing a functional Magnetic Resonance Imaging (fMRI) scan to indicate how often a target word was repeated on screen. The task was divided into two blocks of words, negative and neutral. Contrasts between conditions showed how the "negative" condition correlated with an increase in activity in the dorsal ACC, which the authors interpreted as increased demand to ignore the emotional aspect of the stimuli in favor of a purely cognitive treatment.

As is true for the two previously described structures, studies and models in humans have relied on animal models to guide their inquiries. The issue with the vmPFC is that there is no pure equivalent in the rodent brain. This was highlighted in a review by Allman and Hakeem (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001), which illustrates how the cytoarchitecture of the ACC is unique. Authors modeling fear-conditioning in rodents have found that the functional equivalent of the human vmPFC would be the infra-limbic Prefrontal Cortex (IL-PFC). An important study by Morgan and LeDoux (Morgan & LeDoux, 1995) indicated that lesions to the ventral portion of the medial prefrontal cortex in rats, as opposed to lesions to the dorsal portion, had a detrimental effect on extinction learning but not acquisition of fear. This role was further clarified by a later study using electrolyte lesions of the vmPFC of rats (Quirk, Russo, Barron, & Lebron, 2000). The authors reported that the IL-PFC was necessary mostly for the consolidation and recall of extinction learning, while the expression of extinction was under the amygdala's control. This was further illustrated by recording directly the activity of the cells during

extinction recall in conditioned rats (M. R. Milad & Quirk, 2002). These authors reported that activity of the IL region was specifically related to recall of extinction learning 24 hours after learning, and that the amount of freezing was inversely correlated with the amount of firing of IL neurons. This role in consolidation of fear extinction was further supported by an original study by Santini and colleagues (Santini, Ge, Ren, Pena de Ortiz, & Quirk, 2004) who injected the protein-synthesis inhibitor anisomycin into the ventricles of rats learning extinction, and subsequently observed that it had no effect on short term expression of extinction but that it prevented rats from displaying extinction memory 24 hours later. This study illustrates the necessity of new protein synthesis for the consolidation of extinction learning. Confirming further the role of the vmPFC, the authors replicated this detrimental effect with microinfusion of anisomysin targeting the medial PFC specifically, contrasting with the intra-insular cortex injections.

Based on these studies, investigations of human fear-conditioning have replicated the role of the vmPFC in the consolidation of fear extinction. One study by Kalisch and colleagues (Kalisch, et al., 2006) showed that activation of the left vmPFC was correlated with the recall of extinction. Specifically, they saw that the left vmPFC's activity was correlated with faster reaction time when subjects were presented with the extinction context (defined by the color of the screen) compared to the un-extinguished context. However, the authors did not report any differences in GSR to the extinction context versus the conditioning context. Milad and colleagues (M.R. Milad, et al.,

2007) replicated the finding of the vmPFC's role in recall of extinction. They observed that recall of extinction learning 24 hours after training induced significant activation of the vmPFC, which correlated with a decrease in GCR reactivity to the CS+. This is similar to the negative correlation between GCR reactivity and hippocampal activation following extinction learning (see above). Moreover, this relationship between the vmPFC and extinction was also visible at the structural level. In one study, Milad and colleagues correlated the thickness of the cortex in the vmPFC region, assessed using structural brain imaging, with the level of extinction learning in healthy volunteers (M. R. Milad, et al., 2005).

From these studies, we can conclude that the vmPFC plays an integral role in extinction learning, mostly at the stage of consolidation and recall of the memory. The importance of this process comes into play especially in the case of some disorders like Post-traumatic Stress Disorder, to which we will return to later. But for the healthy individual, there appears to be a balance between learning fear and learning what not to fear that allows for optimal functioning depending on the situation. As is discussed in the review by Zhang and colleagues (Zhang, et al., 2006), defensive behavior and fear must be considered relative to environmental factors. For example, in individuals living in areas that are prone to crime, hypervigilance may not be as much a symptom as it is an adaptive behavior. The same is true for military personnel, who face daily threats during their deployment. The issue may come at a later stage when they are back home in a safe context. The capacity to recognize threats, to act upon them, but also to

recognize signals of safety, is integral to mental health and fear regulation.

This being said, not all situations present equal levels of arousal and can lead to similar levels in terms of learning and memory. Other agents help modulate how well memories are formed and recalled. One of the most potent of these agents is stress and its endocrine correlates.

2. STRESS

2.1 Definition of Stress

Whereas fear seems easily defined, stress is a term that has been applied to many concepts. The term originates from the field of physics and describes the force exerted upon an object. It was integrated into the field of medicine and psychology to describe a repertoire of biological and behavioral reactions that follow the presence of noxious stimuli, be they injuries or toxins (Selye, 1998). The important aspect of this first definition was the non-specificity of the trigger of such reactions. Considering the aversive nature of fear, it can therefore be postulated that fear and stress are related concepts: both concepts deal with a reaction to a threat. However, while fear relates specifically to imminent threats to physical integrity, stress seems to deal with more diffuse threats, to which we will return later. Stress has since become a common expression to describe the hardships of daily life and a variety of psychological and physiological

sensations. In order to best study stress, many models have tried to capture this concept with the best definition. One way to look at stress is to define it as the psychophysiological reactions occurring when demands from the environment exceed the resources possessed by the individual (Lazarus, 1993). In other words, when the dynamics between environment and individual threaten the equilibrium, or homeostasis, the individual will respond with stress (Lupien & Lepage, 2001; McEwen, 2000). There are still two elements to consider regarding that definition: first, what constitutes a threat to homeostasis, and second, is there a common element underlying the reaction of an individual to a threat?

The first element to consider is the nature of the threats to the individual. In his body of works, Selye maintained that the triggers are not specific and may be of various origins. A first possible categorization of triggers, which we shall call *stressors* for the purpose of this work, divides between physiological and psychological stressors. For example, rats would be stressed both by injuries and by being left in an open space, something that rats are eager to avoid. In humans, tasks as various as dipping one's hand in ice-cold water and public speaking are both considered stressors. Dickerson and Kemeny (Dickerson & Kemeny, 2004) have proposed an interesting set of qualities that may constitute a stressor in humans especially. In their meta-analysis of over 200 studies looking at acute stressors, they observed that tasks involving novelty, unpredictability and evaluation by others of their performance, triggered a larger stress response. For the purpose of this work, we will consider these elements as

crucial to the nature of a stressor. Especially in humans, the threat to the ego that comes with social evaluation will be a key factor to our discussion and experimentations described in coming chapters. Thus, an important aspect derived from the work of Dickerson and Kemeny is that, whereas fear is related to an immediate threat to physical integrity, stress can be triggered even in the absence of physical threat. It must also be highlighted that novelty and unpredictability are elements that are commonly found in a fear-conditioning paradigm. This raises the question of fear-conditioning as a potential trigger for a stressful reaction. Or, viewed differently, perhaps the anticipation of a fearful reaction could induce a stress response, especially if the individual is not made aware of what the situation may be. Therefore, at a conceptual level, there is significant overlap between the definitions of fear and stress.

The next element that must be considered is the actual response that qualifies as a stress response. Stress has been shown to affect various physiological and behavioral responses. While stress and fear share the possible activation of the SNS, one system has been observed to cross species and end in the release of an endocrine messenger that underlies many of the stress-related modifications of physiology and behavior. This system, termed the Hypothalamic-Pituitary-Adrenal (HPA) axis, is responsible for the release of glucocorticoids, namely cortisol in humans. We will turn to this system next.

2.2 Anatomy of the HPA-axis

The first and key structure of this endocrine axis is the hypothalamus, a body composed of many nuclei that sits below the thalamus. It is composed notably of the paraventricular nuclei, which are rich in two types of parvocellular neurons, i.e. arginine-vassopressin (AVP) and corticotropin-releasing hormone or factor (CRH/F) neurons (Swaab, Bao, & Lucassen, 2005). The hypothalamus receives input from many important structures such as the amygdala (J. LeDoux, 2003), and hippocampus, as well as from the olfactory cortex and reticular formation (Feldman, Meyer, & Quenzer, 1997). It must be noted here that structures that control the HPA-axis are also part of the fear axis described earlier. Herman (Herman, et al., 2003; Herman, Mueller, & Figueiredo, 2004; Herman, Ostrander, Mueller, & Figueiredo, 2005) has reviewed the seminal work conducted on the hippocampus and amygdala, as well as other central nervous system structures such as the nucleus of the solitary tract (NST), the Raphe nucleus and Bed Nucleus of the Stria Terminalis (BNST). While some structures project directly to the hypothalamus, the hippocampus and amygdala have been shown to be indirect modulators of the HPA-axis activity, especially through glutamatergic and GABAergic projections to intermediate structures such as the BNST. Stimulation of the amygdala has been shown to trigger HPA-axis reactivity as observed in ACTH and corticosterone secretion (Gallagher, Flanigin, King, & Littleton, 1987; Kawakami, et al., 1968; Matheson, Branch, & Taylor, 1971; Redgate & Fahringer, 1973). This further indicates potential overlap between the reactivity to fear and stress. All these structures

may provoke the release of CRF through excitatory input or through double inhibition (GABA-GABA). This release of CRF into the portal system of the pituitary will activate CRF receptors located in the anterior portion of the pituitary gland, which sits directly below the hypothalamus (Pritchard & Alloway, 1999). This gland is one of the rare areas of the central nervous system that has direct access to the blood stream, without the protection of the blood-brain barrier (BBB). Thus, when stimulated, the pituitary will directly release adrenocorticotropic hormone (ACTH) into the blood stream. Once ACTH reaches the adrenal cortex located on top of the kidneys, it binds to ACTH receptors of the zonae fasciculata to provoke the synthesis and release of glucocorticoids (GCs) into the blood stream. Cortisol (corticosterone in animals) is the main GC released in response to stress and has been the principle outcome measure in stress research for the past years. It is a lipophilic hormone derived from cholesterol that has been shown to affect the metabolism of glucose (Baron, Wallace, & Brechtel, 1987; Morton, 2010), the immune system (Rickard & Young, 2009; O'Donovan, et al., 2010), the cardiovascular system (Rickard & Young, 2009), body temperature (Chowers, Conforti, & Feldman, 1968) and other organic tissues (for complete review, see Chrousos, 2009). Cortisol can also cross the BBB and bind to receptors located on neurons of various regions of the central nervous system, notably regions of and connected to the HPA-axis, to modify the activity of these regions and also shut off further release of cortisol by a negative feedback loop. These receptors are of two kinds: mineralocorticoids, which are the most sensitive to binding, and glucocorticoid receptors, which are much less sensitive to the

presence of cortisol. Before we get to the interaction between cortisol and the brain, we must underscore that the HPA-axis and cortisol are a slow system. Typically, it can take up to 30-40 minutes for cortisol to reach its peak concentration after the onset of a stressor (Kirschbaum & Hellhammer, 1994). This delay is important to keep in mind when we review the studies investigating the effects of stress on the brain. Also, it must be kept in mind when contrasting with fear, which is known to lead to fast reactions of the SNS.

While cortisol receptors are present in many brain regions, it is interesting to note that they are highly expressed in regions linked to fear-conditioning, namely the amygdala, the hippocampus and the vmPFC (Herman, et al., 2003; Herman, et al., 2005). Studies on the impact and function of cortisol in these brain areas illustrate the various methods used to assess cortisol levels, and highlight what these methods can teach us as well as what limitations they suffer from. However, before we explore the ways to sample cortisol, we must first turn to an important aspect of this hormone, namely its chronobiology and reactivity.

When assessing cortisol, one can either look at the basal secretion over a period of time or one can look at the secretion of cortisol following a specific event, i.e. reactive cortisol. These two measures afford a different perspective on the integrity and function of the HPA-axis. Cortisol is known to follow a diurnal cycle, which begins with a peak in the morning, termed the Cortisol Awakening Response (CAR), and declining throughout the day. The presence of a

cycle offers various avenues of research, such as studying the slope of the decline throughout the day or measuring the magnitude of the CAR. This last measure has been widely recognized as a good proxy for the integrity of the HPA-axis, since it seems to be mostly independent from any input from higher regions of the central nervous system (Herman, et al., 2005; Kudielka & Wust, 2010), although some studies have reported a link between the volume of the hippocampus and magnitude of the CAR (M. Pruessner, Pruessner, Hellhammer, Bruce Pike, & Lupien, 2007). The CAR is regarded conceptually as a marker of the total capacity of the HPA-axis to mount a response when faced with a trigger. Hence, while it is considered an index of basal cortisol levels, it is also partly a reactive measure triggered by the anticipation of the day's events to come. Therefore, the CAR may be a significant tool to measure and analyze the general integrity of the HPA-axis.

The other type of measure, reactive assessments, is used mostly to illustrate the magnitude of cortisol secretion that follows a stressor. This can be looked at either as a general secretion (global amount including the baseline level) or as an increase relative to each individual's baseline level. These measures are instructive in terms of the sensitivity of the system to challenges, whether they are physical challenges such as the Cold Pressure Task (Lovallo, 1975), or social challenges such as the Trier Social Stress Task (Kirschbaum, Pirke, & Hellhammer, 1993). A further interest lies in the comparison of these two measures in order to establish a pattern that may provide a clearer picture of the HPA-axis function and regulation. For example, a lack of cortisol increase in reaction to a stressor combined with a low

or absent CAR might indicate a systemic problem in the HPA-axis. Alternatively the presence of a normal CAR instead could indicate that the stressor was too mild and may not have been enough to trigger the HPA-axis. These distinctions may be very important for studies that include stress as a modulating factor, such as ones using fear-conditioning paradigms. In the following section, we will turn to the various protocols that allow sampling and measurement of cortisol in humans, including their strength and limitations, which become crucial when discussing the studies of the influence of cortisol on fear-conditioning.

2.3 Measuring cortisol

Various methodologies have been used to assess the levels of cortisol, whether in rodents or in humans. Animal studies have usually examined the plasma or cerebrospinal fluid (CSF) concentration of the hormone. In human studies, however, three methods have generally been used. The first method, similarly to that used in rodents, is to assess the plasma concentration of cortisol by simple blood collection. This method has the advantage of providing a clear measure of the total amount of cortisol present in the system. It must be noted that some of the hormone is at times bound by cortisol-binding globulin (CBG) and serum albumin (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007). According to the free hormone hypothesis, the amount of cortisol bound to proteins is considered to be biologically inactive, but can be released from these proteins when the demand exceeds the quantities that are free. Hence, assessing the levels of both free and

bound cortisol gives a good index of the active as well as potentially active cortisol in the system. The activity of CBG and serum albumin may also in part explain the varying concentrations of free cortisol and therefore inter-individual differences in basal cortisol levels. The limitation of this method in human studies comes from the context where blood can be drawn, which requires trained professionals. Furthermore, it is limited also by the fact that blood from humans cannot be repeatedly drawn, which limits the capacity to establish dynamic changes in the concentration levels across a short period of time. Lastly, the act of drawing blood can be itself a stressor, which may induce a significant stress response in some individuals. The DSM-IV recognizes trypanophobia, the intense fear of medical procedures involving needles, as a sub-class diagnosis of simple phobias, which is estimated to affect roughly 10% of the population (APA, 2004). While the exact prevalence of this specific phobia is not known, it may affect studies with larger samples.

A second way to measure the activity of the HPA-axis is through the concentrations of cortisol present in urine. This method allows for the computation of the total excretion of filtered free cortisol over period, most often of 12 hours. This can be useful when assessing the integrity of the HPA-axis or if investigating pathologies that would chronically affect the production of cortisol over the course of a day or night, such as Cushing's syndrome. It has the advantage of being less invasive than plasma cortisol and to be collected outside of a laboratory. However, it does suffer from a similar limitation as blood samples, namely that it cannot be collected repeatedly in short periods

of time. This, again, limits the capacity to investigate the dynamics of the HPA-axis. Another possible confound is the presence of metabolites of cortisol in urine, which may affect the precise assessment of concentrations of cortisol itself. Last, urinary free cortisol may suffer more from time-induced decay compared to plasma, which requires greater care in the manipulation and storage of samples.

A third method of collecting cortisol is through the saliva, since free plasma cortisol crosses passively into the saliva glands. This approach has been extensively reviewed (Levine, et al., 2007) and affords an accurate index of the actual concentrations of free cortisol in the plasma. The main advantage of salivary cortisol as a method of collection is that it is completely non-invasive. Generally, participants in a study will be asked to keep a small cotton swab inside their mouth for 30 to 60 seconds. This is usually enough to collect the appropriate amount of saliva. This also allows easy collection of samples outside the laboratory context, in more natural settings such as at home or at work. Furthermore, this method makes possible the collection of multiple samples in a short period of time, allowing for the description of the dynamics of the HPA-axis, from measuring the basal activity to assessing the stress response to an acute stressor. The limitations for this measure are mostly related to saliva and the mouth: many factors such as food particles, lack of saliva, or bloody gums can all affect the concentration of cortisol in saliva samples. Hence, if participants are not clearly instructed on the exact procedure for sampling or do not comply with these instructions, the quality of the

samples may be compromised, leading to incorrect values. However, if the instructions are clearly stated and followed, saliva samples allow for reliable measurements. Furthermore, samples can be kept at room temperature for a certain amount of time, even days, before the cortisol is degraded, allowing enough time for participants to return the samples to the investigator.

2.4 Fear and Stress: the effect of stress on fear-conditioning

Given the pleitropic activity of cortisol, its influence on the cardio-vascular system and its ability to cross the blood-brain-barrier to reach receptors in the central nervous system, some investigators have looked at the effects of cortisol on fear-conditioning paradigms. There are two main moments where cortisol can be manipulated efficiently to affect fear-conditioning: pre-training and post-training. In one study, Cordero and colleagues (Cordero, Kruyt, Merino, & Sandi, 2002) used the corticosterone inhibitor metyrapone injected subcutaneously in rats prior to training in a context-based fear-conditioning paradigm. They observed that the absence of corticosterone led to a decreased fear response 24 hours after training for both doses of 50mg and 100mg/kg. At 7 days post-training, they replicated this decrease in freezing for the higher dose of metyrapone only. Furthering this finding, Hubbard and colleagues (Hubbard, Nakashima, Lee, & Takahashi, 2007) also showed the importance of glucocorticoids in the formation of contextual fear memories by injecting a CRF_1 receptor antagonist in the basolateral and central nuclei of the amygdala of rats trained in a context-based

fear conditioning paradigm. They observed that, two days after training, the inactivation of the CRF receptors decreased the percentage of freezing in a dose-dependent manner. Furthermore, this effect was only seen if the CRF_1 antagonist was injected into the basolateral nuclei, but not the central nuclei. However, this role of cortisol in increasing the strength of consolidation was contradicted by the results from Skorzewska and colleagues (Skorzewska, et al., 2007). They observed that, when injecting subcutaneously rats with corticosterone 90 minutes pre-training, rats showed a decreased freezing response 24 hours after training. This was accompanied by an increase in c-fos concentration in the cingulate area, indicating an increase in gene transcription for this area. A first difference between the two previous studies that may explain the conflicting results is that, while Hubbard and colleagues targeted CRF, which is released early in HPA signaling, Skorzewska and colleagues injected the rats with corticosterone, the end product of the HPA-axis. While CRF is a promoter of the stress response, one of the roles of corticosterone is to bind to receptors in the central nervous system and thus shut down the activity of the HPA-axis. Another difference is the site of injection. While Hubbard and colleagues used intra-amygdalar injections, Skorzewska injected corticosterone subcutaneously. This may have influenced the action at the central level because of the smaller concentrations reaching the amygdala itself. Thus, the picture for pre-training manipulations of glucocorticoids remains unclear and needs further investigation (Rodrigues, et al., 2009).

Manipulation of GCs after training has been the subject of additional studies. Quirarte and colleagues (Quirarte, Roozendaal, & McGaugh, 1997) have shown that, while GCs post-training increased the retention of inhibitory avoidance, this effect was dependent of the activity of norepinephrine (NE) in the basolateral nuclei of the amygdala. Using NE antagonists, they abolished the increase in retention of latencies 48 hours after acquisition that was provoked by post-training administration of dexamethasone, a synthetic glucocorticoid. This enhancing effect of post-training GC on long-term memory was replicated by Roozendaal and colleagues for contextual avoidance (Roozendaal, Nguyen, Power, & McGaugh, 1999; Roozendaal, de Quervain, Ferry, Setlow, & McGaugh, 2001), as well as auditory fear-conditioning (Hui, et al., 2004; Marchand, et al., 2007), and taste aversion conditioning (Miranda, Quirarte, Rodriguez-Garcia, McGaugh, & Roozendaal, 2008). However, while effects seem clear for long-term memory, the same manipulations do not seem to affect short-term memory (Rodrigues, et al., 2009).

Based on these studies, researchers have examined the effects of manipulating cortisol in human studies of emotional memory and fear-conditioning. These studies can be divided into two main categories. A first category has investigated the influence of stress on declarative verbal memory. Buchanan and colleagues (Buchanan & Lovallo, 2001) used images drawn from the International Affective Picture System (IAPS) in a study of free recall in young healthy men and women. Subjects were divided into a Stress (hydrocortisone) or Control (placebo) condition and were asked to look at pictures with

positive, negative or neutral valence. One week later, subjects were asked to recall the pictures encoded while under the effect of stress or placebo. Their results indicated that in general, stress improved the number of images recalled compared to placebo. Furthermore, the effect was stronger for the emotional pictures compared to the neutral ones, independent of the actual valence of the pictures. The authors interpreted this as an enhancing effect of stress of emotional memory through the action of GCs. Cahill and colleagues replicated this effect using post-encoding manipulations of endogenous cortisol levels (Cahill, Gorski, & Le, 2003). Their study used ice-cold water to induce an endogenous increase of cortisol levels and warm water as a control condition, as well as IAPS pictures for encoding, both arousing and neutral. One week post-learning, they observed that recall of the pictures in general showed a trend for better performance in the stress group. This effect became significant when the investigators looked at the arousing material compared to the neutral pictures: the stress group showed better recall to the emotional material. Interestingly, this effect was markedly stronger in female subjects compared to male -however, this may have been due to the higher number of female subjects in their study (total of 34 out of 48). Surprisingly, there was no correlation between the cortisol levels and the performance on recall. This may have been due to inter-individual differences in cortisol output or the absence of control over the stage in the menstrual cycle in the female subjects. This influence of menstrual cycle over the cortisol secretion in the context of stress was illustrated by Kirschbaum and colleagues (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). They have shown that the

phase of the menstrual cycle and use of oral contraceptives can modify the cortisol secretion in stressed women. Salivary cortisol was higher after stress in women in the luteal phase, while plasma cortisol was higher in females using oral contraceptives, compared to women in follicular phase and men.

Furthering the investigation of the effects of cortisol and NE on emotional memory, Maheu and colleagues (Maheu, Joober, Beaulieu, & Lupien, 2004) measured the effects of metyrapone (cortisol inhibitor) and propranolol (NE receptor blocker) on both short and long-term memory of an emotional story displayed as a succession of slides, the first and third tier being neutral while the second tier contained emotional material. In a first study, they observed that, while propranolol and metyrapone had no effects on short-term memory, metyrapone decreased levels of salivary cortisol while propranolol increased the levels of cortisol. At a one-week post-learning recall test, however, they observed that the metyrapone group showed decreased performance compared to the propranolol and control groups, supporting the role of cortisol for the consolidation of memory, though this effect was true for both neutral and emotional material. When they increased the dose of propranolol (80mg versus 40mg in the first study) and compared it to a placebo only, the authors observed a decrease in recall for both short and long-term memory of emotional material only. This indicated that both NE and cortisol might play a role in the formation and consolidation of emotional memories. The same authors published another report investigating the effects of psychosocial stress in male subjects divided into three groups:

metyrapone, propranolol and placebo (Maheu, Joober, & Lupien, 2005). Using the Trier Social Stress Task (TSST; Kirschbaum, Pirke, et al., 1993), a public speaking task, before encoding, they observed that both the placebo and propranolol group showed greater recall at short-term, but that there was no effect of drug one week after initial learning. It must be noted that this study used a purely neutral story, suggesting that cortisol may present an effect specific to emotional material, consistent with the work by Cahill and colleagues. The same year, Kulhmann and colleagues (Kuhlmann, Piel, & Wolf, 2005) manipulated cortisol levels before recall to examine how stress may impact memory with emotional versus neutral valence. Specifically, 24 hours after encoding, stress was induced in subjects by performing the TSST prior to a recall test of emotional and neutral words. The authors reported that, while stress had no effect on digit span, attention and psychomotor speed, it decreased the performance on recall for arousing words (negative more than positive) compared to neutral words. Thus, while increased cortisol during or immediately after encoding might enhance recall of emotional material, an increase in cortisol prior to recall may impair performance by interfering with retrieval of information. However, these studies asked subjects to encode and recall verbally emotional material, a task that recruits many regions of the central nervous system that may not be involved or necessary for fear-conditioning. Furthermore, while fear-conditioning is assessed mostly through peripheral indices, the preceding studies have mostly avoided looking at peripheral measures. Only Maheu and colleagues (Maheu, et al., 2004; Maheu, et al., 2005)

measured heart rate and blood pressure, specifically to assess the impact of propranolol.

The second category of studies investigating the effects of stress has specifically looked at fear-conditioning tasks. The first published report came from Zorawski and colleagues (Zorawski, et al., 2005), who investigated the effect of basal levels of cortisol on fear-conditioning acquisition and retention in a 24 hours delay extinction test. The first objective of their study was to examine if a simple fear-conditioning paradigm could induce a significant cortisol increase such as the one observed in animal studies. Their results showed the contrary, with levels of cortisol decreasing throughout the task. This effect could be due to the natural circadian rhythm of basal cortisol secretion. They then divided their subjects into two groups using a median split of the cortisol levels 45 minutes after the conditioning procedure. They observed that, in males only, the subjects with higher levels of cortisol showed greater GSR to the CS+ compared to the CS-. The effect was not present for males with low cortisol and for females. It must be noted however that because the “high” and “low” cortisol groups were determined by a median split they do not represent significantly higher or lower values relative to a regular baseline. Rather, they might be interpreted as more or less decrease in basal cortisol throughout the task, either due to the fear-conditioning task or to inter-individual differences in basal HPA-axis circadian rhythm. Furthermore, the authors recruited both males and females, but did not control for the stage of the menstrual cycle in the female subjects, with roughly half of them using oral contraceptives. This

absence of control may have influenced the levels of cortisol in female subjects and might explain why there was no significant effect of cortisol on GSR. Lastly, the authors reported no sex differences on the effect of cortisol on the 24 hours post-acquisition retention test (extinction paradigm). Therefore, their results did not support an effect of cortisol on the consolidation of pavlovian fear-conditioning and contrasted with the literature investigating verbal declarative free-recall of emotionally arousing material mentioned above.

Following up on their first report, Zowarski and colleagues (Zorawski, et al., 2006) published a follow up study that used post-acquisition induction of cortisol secretion through the use of a psychosocial stress task. Replicating their design from the first study, they used a median split to create "high" versus "low" cortisol groups across their subjects (Zorawski, et al., 2005). They observed that, in males, there was a significant effect of post-acquisition cortisol (+45 minutes) on GSR response to the CS+ versus CS- in the high cortisol group only at late acquisition, while there was no effect at the extinction stage 24 hours after acquisition. The authors highlighted the interaction between stress, sex and acquisition of fear-conditioning, since only males displayed that interaction between cortisol levels and differential fear-conditioning. However, once more, there was no effect on long-term memory of the conditioning.

The same year, Jackson and colleagues published a report on their study of cortisol and fear-conditioning (Jackson, et al., 2006). They tested young healthy undergraduate males and females by first

having them undergo a psychosocial stress task, i.e. the TSST with panelists outside the room, or a control condition, followed by a 60 minute recovery period where participants were asked to sit quietly and listen to soothing music. Following this rest period, subjects were asked to undergo a fear-conditioning paradigm using faces as a CS and a high-pitch scream as US. Subjects were presented 8 CS paired with the US, followed by a single presentation of the CS alone, which served as a measure of conditioning. Their results showed that, in males only, prior exposure to stress led to an increase in reactivity to the CS+. Females did not show this increase in reactivity. They interpreted these findings as a facilitation of fear-conditioning by stress in males, illustrating again the sexual dimorphism in reactivity to stress and fear observed previously by Zorawski and colleagues. It must be noted that in this study, subjects did not have increased cortisol levels during the acquisition stage, which took place 60 minutes following exposure to the stressor. Cortisol levels usually peak 30 minutes after the onset of a stressor and return to normal levels roughly 60 minutes after the onset of a stressor, the exact timing of the acquisition task in this study. The other main limitation comes from the single recording of GSR to the CS+ without the US, which does not provide information as far as the rate or strength of acquisition compared to initial levels. Therefore, it is hard to conclude on any influence of pre-training cortisol modulations on fear-conditioning from this study.

In another line of investigation, Stark and colleagues (Stark, et al., 2006) studied the effect of hydrocortisone or placebo on fear-conditioning in young males and females in a functional MRI paradigm.

For this study, the CSs were geometrical figures paired with a mildly painful electrical shock co-terminating with the CS+. The authors reported that males treated with hydrocortisone showed decreased differential conditioning compared to the placebo group during the first interval response (0 to 5 seconds of the 8 seconds of CS). Hydrocortisone had no effect in females on this differential conditioning. Furthermore, they reported that presentation of the CS+ was associated with greater activation of the anterior cingulate cortex, right hippocampus, hypothalamus, left lateral orbitofrontal cortex as well as ventromedial orbito-frontal cortex. This activity was increased in males of the hydrocortisone group, while females of the hydrocortisone group showed decreased activity. This study therefore illustrated how pre-training manipulation of cortisol levels may impair acquisition of fear-conditioning, possibly through facilitating the activity of regions involved in extinction such as the hippocampus and anterior cingulate cortex. This study was followed by the report of Tabbert and colleagues (Tabbert, et al., 2010), who used a similar design in a sample of female subjects only to investigate the effect of hydrocortisone on acquisition as well as extinction using fMRI. Replicating the findings of the previous study, they found no effect of hydrocortisone on acquisition of fear-conditioning. However, when comparing groups at the extinction phase that followed immediately the acquisition phase, they observed increased GSR to both CS+ and CS- in the hydrocortisone group. Interestingly, the brain regions activated in response to the conditioning task changed depending on the contrast. When the contrast was CS+ > CS-, the activity was increased for the thalamus, right ACC, left hippocampus and left

insula; when the contrast used was CS- > CS+, there was increased activity in the thalamus, right insula, left amygdala, right hippocampus and right ACC. The authors pointed to the differential activation of regions depending on the reactivity to stimuli during the acquisition phase. However, it is interesting to note that, once more, pre-training manipulation of cortisol did not yield significantly different levels of reactivity to the CS+ compared to the CS-. It must also be noted that the previous two studies were the first to recruit females that were all using oral contraceptives in order to control for the levels of gonadal hormones, especially estrogen, which has been found to modulate the effects of cortisol.

In sum, whereas studies in rodents all seem to indicate that glucocorticoids play an important role in the consolidation of fear-conditioning, especially in the case of context-based conditioning, human studies show another pattern. Studies on verbal recall of emotional material, a task that is highly dependent on the involvement of the hippocampus, similar to context-based conditioning in rodents, show that an enhancement of the consolidation of emotional information and a potential impairment of recall are both caused by increases in cortisol. In the case of pavlovian fear-conditioning, which does not rely on the hippocampus as much, the picture is less clear. Even though the amygdala supports this type of learning, even if it does project to the hypothalamus to trigger an HPA-axis response, it is unclear if a simple pavlovian fear-conditioning paradigm can induce a stress response. Moreover, the impact of an increase in cortisol prior to training seem to affect the general galvanic skin level, independent

of the stimulus. It may be that pre-training cortisol increases arousal without allowing for better discrimination between stimuli. As for post-training manipulations, it does not appear that elevated cortisol levels significantly increased the strength of conditioning. One additional interesting fact that emerges from these studies is the systematic sexual difference in GSR, something that may be accounted for by the general lack of control for the stage of the menstrual cycle in female subjects.

3. SUMMARY

The review of fear-conditioning and stress raises important questions that provide the rationale for studies described in the first two chapters of this thesis. First, despite evidence from animal studies on the role of glucocorticoids in learning of fear through conditioning, as well as evidence from human studies on emotional declarative memory, it is still unclear how cortisol levels relate to fear-conditioning in healthy humans. Considering the effects of cortisol on important structures like the amygdala-hippocampus-ACC network, it seems surprising that so few studies have measure GSR and cortisol concomitantly. One question that has not been addressed systematically is: since fear and stress share common situational elements, are both a reaction to a threat and share biological systems, is fear-conditioning a sufficient paradigm to induce an HPA-axis response? This was the first objective of this thesis. A second line of research that has been poorly investigated is the effect of post-training endogenous cortisol manipulations on immediate extinction. It

must be remembered that the only study that manipulated post-training cortisol level tested extinction only 24 hours later. Thus the effect of stress on fear-conditioning, while apparently clearer in animal studies, is not understood in humans. Therefore, the second objective of this thesis was to investigate the effects of cortisol on immediate extinction learning. This design would allow for a better understanding of the process of consolidation of emotional memory. Another important reason to investigate the interaction between fear and stress comes from the field of psychiatry, especially when considering PTSD. As we will explore in the second half of this introduction, as well as in chapter 3 and 4, the interaction of fear and stress is crucial in our understanding of the pathophysiology of PTSD and may help us go beyond the current model.

4. POST-TRAUMATIC STRESS DISORDER: a tale of fear and stress

4.1 Definition of PTSD

PTSD is a DSM-IV anxiety disorder (APA, 2004) that was first introduced in the third version of the DSM. Even though it wasn't defined as such before, it was recognized already in military circles as shell shock during the First World War and later as Combat Fatigue. Even today, military personnel define it more often as Operational Stress rather than by PTSD. The main change that occurred with the advances in psychiatric research was a broader definition that includes various trauma types instead of purely military-related incidents.

Previous epidemiological studies (Breslau, 2001; Breslau, et al., 1998) have indicated that, while a majority of the population will be exposed at one time in their life to a traumatic event, only a fraction of those individuals will develop symptoms of PTSD. The other crucial element that can be concluded from epidemiological studies is that the number of individuals suffering from PTSD decreases over time following the traumatic event, either through therapy or through natural remission. Whereas roughly 15% of individuals exposed will suffer from symptoms one month post-trauma, only 7% develop chronic PTSD at the 3-months mark. In the first 12-18 months, this number falls to 2-4%. Studies have therefore tried to identify the factors that may explain the persistence of symptoms as well as those that explain the resilience to the impact of trauma observed in many individuals. Before we turn to the model used to study PTSD and review the studies investigating the pathogenesis and maintenance, we will briefly review the diagnosis as it is established in the most recent version of the DSM.

4.2 PTSD Diagnosis: a triad of symptoms

The diagnosis of PTSD requires a first criterion to be fulfilled, namely the exposure to a traumatic event (A1); exposure in this case defines either a direct victim or witness of the event. The list of possible events as described in the DSM-IV contains 13 types of events, ranging from natural disasters (e.g. earthquake or tsunami) to physical assault or sexual assault. It must be noted that the incidence of PTSD following different types of events is not equal. Events that involve another human being as a perpetrator or as a threat usually

leave the victims with worse symptoms compared to events where the agent is not under human control, such as a natural disaster or accident. Furthermore, at the time of exposure, victims must have experienced significant fear, horror or helplessness (criterion A2). This feeling of terror is thought to be the root cause of the disorder. The next three clusters of the diagnosis describe the actual symptoms that may afflict the survivor of the event.

The first cluster of symptoms (Cluster B) falls under the definition of Flashback and Intrusive Memories. This describes the occurrence of either complete sensory reliving the event without any cues from the environment triggering the feeling (finding one's self trapped in a complete hallucination of the event) or of un-cued and undesired memories of the event (e.g. hearing the noise produced by folding of the metal of a crashing car or smelling the distinct odor of airbags deploying). It also describes the frequent occurrence of nightmares about the traumatic event, as well as intense psychological distress and physiological reactivity to reminders or symbols related to the event. The key element of this cluster is that these memories do not seem to be triggered by any environmental cues; are psychologically painful and seem to completely escape the conscious control of the individual. Also, these memories are usually only partial and fragmented and focus on basic sensory elements and are usually not complete with spatial-temporal context.

The second cluster of symptoms (Cluster C) describes the general state of emotional numbing and the adoption of avoidance-

based behavior by the individual. In other words, the person suffering from PTSD displays a significant lack of affect and emotion and loses interest in previous activities. This is reminiscent of the anhedonia observed in major depression. At the same time, the individual will avoid any reminder of the event, whether they are physical locations or objects or even conversations touching the subject of the event. This cluster highlights the general social withdrawal that is displayed by individuals with PTSD, which lessens the probability of help-seeking behavior, inducing a downward spiral of suffering and symptom maintenance. The isolation behavior also has repercussions on loved-ones and families: as they find themselves unable to reach the person suffering, helplessness increases and puts additional strain on relationships.

The third and final key cluster (Cluster D) focuses on the hypervigilance displayed by individuals suffering from PTSD. Specifically, this cluster describes how autonomic functions are altered, from an increase in general startle reactivity to difficulties falling or staying asleep. Also, part of this cluster describes how an individual suffering from PTSD will see threatening elements in his environment where there are none and will misinterpret safety cues or ignore them completely. Finally, a hallmark of this cluster that may also contribute to the social isolation following the trauma is the presence of bursts of anger and general irritability. The important aspect of this cluster is the role of the autonomic nervous system in the general feeling of hyperarousal, increased startle response and

altered sleep pattern, something which we will discuss in the section on the model of PTSD.

The diagnosis therefore shows us that PTSD is a fear-based disorder that emerges as a consequence of a terrifying event. While some of the symptoms are directly tied to the event in the form of memories and directly under control of the central nervous system, symptoms of the third cluster involve both central as well as peripheral factors to create the feeling of hyperarousal. Moreover, the social aspect of the disorder contributes to the general distress and may bear special importance in the maintenance of symptoms, considering its impact on help-seeking behavior. This latter aspect does not clearly fall into a strictly fear-based set of behavior and may be closer to other emotions of greater social relevance than fear. Keeping in mind this wide variety of elements involved in the diagnosis of PTSD, we next turn to the model used to study this complex disorder.

4.3 Modeling PTSD

Since its inclusion in the DSM-III, PTSD has been the subject of many studies using various approaches. Most of these studies however, are based on the model presented by Pitman (R. K. Pitman, 1989; R.K. Pitman, Shin, & Rauch, 2001), which uses the principles of fear-conditioning to describe the traumatic event and post-traumatic reactions. In this model, the traumatic event itself is seen as fear-conditioning where various elements of the situation are paired up with

the actual threat. The trauma itself would trigger the release of catecholamines and stress hormones such as cortisol, which would create an over-consolidation of the fear memory. This would lead to the intrusive memories, flashbacks and nightmares that are a hallmark feature of PTSD. It would also lead to conditioned responses in the form of avoidance behavior and general hyperarousal. This model is a useful tool to describe the pathogenesis and the origin of the symptoms related mostly to memory. Furthermore, this model allows for the investigation through animal studies, since fear-conditioning, as we illustrated in the first section of this introduction, translates well between species. Based on the neurological studies of fear-conditioning, clear hypotheses on the neurological bases can be established and tested.

Despite these benefits, this model shows some limitations. First, it posits that intrusive memories are the key symptom that differentiates PTSD from other disorders such as generalized anxiety or panic disorder. However, recent studies have shown that clusters C and D may contribute more to the distress of individuals suffering from PTSD then from these intrusive memories (Engdahl, Elhai, Richardson, & Frueh, 2010; Shea, Vujanovic, Mansfield, Sevin, & Liu, 2010). A second limitation is that it posits that release of stress hormones at the time of trauma is necessary for the over-consolidation of the fearful memories. As we will see in the coming section, data accumulated from studies on cortisol levels in PTSD paint a picture that is less than clear. A third limitation of the model lies in the actual definition of the traumatic experience. Some examples of

traumatic experience, such as being victim of an assault or surviving a natural disaster, are temporally and spatially isolated. On the other hand, other traumas such as being present in a war zone or being a victim of repeated abuse during childhood go beyond a specific context and represent a constant threat during a long period of time. This difference may alter the impact of the trauma on the biological responses. Furthermore, traumas occurring at different stages of life may alter the natural course of development and aging of biological systems, something that is not accounted for in the current model. These limitations must therefore be kept in mind when we review the studies that have investigated the potential biological markers of PTSD, to which we will turn in the following section.

4.4 Neurobiology of PTSD

For the past fifteen years, advances in brain imaging techniques, especially MRI, have helped researchers investigate the neurological bases of mental and psychiatric disorders such as PTSD. Following neuropsychological studies in both human and animals, specific structures have been identified to contribute to the symptoms of PTSD. The first report using MRI to investigate the brain integrity of patients suffering from PTSD was published by Myslobodsky and colleagues (Myslobodsky, et al., 1995). These authors found a decrease in signal intensity of the septo-callosal junction in patients with combat-related PTSD, indicating a greater concentration of CSF in the area. However, since this was mostly an exploratory study, the

authors did not report significant quantification and therefore statistical measures of the difference.

This study led to a series of studies investigating the volume of the hippocampus in patients with chronic PTSD. Originally based on the observed deficits in verbal declarative memory in patients (J.D. Bremner, et al., 1993) and the glucocorticoid cascade hypothesis (Sapolsky, Uno, Rebert, & Finch, 1990), Bremner and colleagues investigated the volume of the hippocampus in combat-related PTSD (J. Douglas Bremner, et al., 1995) and victims of childhood abuse (J. Douglsd Bremner, et al., 1997). Both reports highlighted a significant difference in hippocampal volume that was attributed to the neurotoxic effect of constant glucocorticoid exposure. It must be noted however that neither report actually investigated the levels of circulating cortisol levels in their subjects. Furthermore, the first report found an 8% difference in the right hippocampus whereas the second report found the difference in the left hippocampus. The relationship between PTSD and smaller hippocampi was additionally blurred by the high prevalence of comorbid disorders associated with brain abnormalities such as alcohol abuse and depression. Despite these limitations, other studies replicated the original findings of smaller hippocampal volume associated with PTSD (J. D. Bremner, Vythilingam, Vermetten, et al., 2003; Carrion, Weems, & Reiss, 2007; Gurvits, et al., 1996; Hedges, et al., 2003; R. J. Lindauer, Olff, van Meijel, Carlier, & Gersons, 2006; R. J. L. Lindauer, et al., 2004; Pavic, et al., 2007; Villarreal, et al., 2002; Vythilingam, et al., 2005; Wignall, et al., 2004). Confirming this effect, two recent meta-analyses have highlighted the importance of this

phenomenon across multiple studies (Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005; Smith, 2005), while there are also numerous other studies who have failed to observe this difference (Bonne, et al., 2001; De Bellis, Hall, Boring, Frustaci, & Moritz, 2001; J.A. Golier, et al., 2005; Jatzko, et al., 2006; Schuff, et al., 2001; Tupler & DeBellis, 2006; Winter & Irle, 2004; Woodward, Kaloupek, Streeter, Kimble, et al., 2006). There are multiple factors that may explain the discrepancy in the literature, such as the time since trauma, severity of trauma and PTSD symptoms, age of participants, levels of comorbid depression or alcohol abuse. The fact remains that most studies point towards an association between smaller hippocampal volume and chronic PTSD. The exact nature of the relationship remains to be further explored.

These studies echo the findings concerning the role of the hippocampus in acquisition of fear-conditioning as well as extinction learning. In the previous section, we have seen how the hippocampus, through its role of encoding context, may play a role both in triggering the fear response as well as supporting extinction of the same fear response. Furthermore, most of the studies have interpreted their results as a consequence of suffering from chronic PTSD. Wignall and colleagues were the only team to find a significant difference in hippocampal volume in recent-onset PTSD. This finding is contradicted by the data from Bonne and colleagues, the only other study that has investigated a sample exposed to a recent traumatic experience and that found no difference in the volume of the hippocamnpus. One landmark study by Gilbertson and colleagues (Gilbertson, et al., 2002)

added a new dimension to the debate. They found smaller hippocampal volume in identical unexposed twins of Veterans from the Vietnam War, compared with volumes in twins of exposed Veterans who did not develop PTSD. They argued that a smaller hippocampus would represent a risk factor rather than a consequence of suffering from PTSD. In other words, a smaller hippocampus would be unable to fully acquire and process contextual information that is essential for extinction learning; PTSD would therefore be a constant failure to suppress the fear response (CR in terms of fear-conditioning). This proposed role fits well when considering that flashbacks and intrusive memories are independent of context. However, it must be recalled that the hippocampus is involved both in the acquisition of conditioning by providing contextual information, as well as extinction learning. This dual role complicates the debate over the exact role of the hippocampus in traumatic memories.

Based on the fear-conditioning model, another avenue of study has investigated the level of amygdala activity in patients suffering from PTSD. In this area, the picture seems clearer than for the hippocampus. A first report using Positron Emission Tomography (PET) showed that, in a symptom provocation paradigm, PTSD was associated with an increase in activity of the amygdala (Rauch, et al., 1996). Despite the absence of a control group, this was a first study to highlight the importance of the amygdala in patients with PTSD. The interest of the symptom provocation paradigm is that it is a clear translation from exposure to the CS+ in fear-conditioning by re-exposing the subject to elements associated with the trauma, such as

helicopter sounds for veterans. This finding was replicated in a subsequent study by Shin and colleagues (Shin, Kosslyn, McNally, & Alpert, 1997), who found that mental imagery of the trauma induced significantly greater activity in the right amygdala in veterans with PTSD compared to healthy veterans. Interestingly, they did not find this difference when subjects were directly exposed to combat-related stimuli. Later, Liberzon and colleagues, used Single Photon Emission Computed Tomography (SPECT) to investigate the reactivity to combat-related sounds compared to white noise in individuals with and without combat-related PTSD, as well as healthy unexposed subjects. In addition to the brain imaging data, they recorded galvanic skin levels in their subjects across the two stimuli. They observed greater amygdala activity in response to combat noise than white noise in the PTSD group compared with the two other groups. This also translated into greater GSL to the combat-related condition. This observed greater activity of the amygdala to symptom relevant stimuli has since then been replicated in various studies [e.g. Driessen, et al., 2004; Hendler, et al., 2003; Pissiota, et al., 2002; Protopopoescu, et al., 2005; Shin, et al., 1999; Shin, Orr, et al., 2004].

Extending beyond stimuli related to the trauma *per se*, Rauch and colleagues investigated the activity of the amygdala to stimuli that are known to trigger an automatic reaction in the amygdala, namely fearful faces (Rauch, et al., 2000). Specifically, they showed that in a sample of veterans suffering from PTSD, the relative reactivity of the amygdala to masked fearful versus neutral faces was greater compared to the combat-exposed veterans without PTSD. The

interpretation of this finding would be that the amygdala is hypersensitive in individuals with PTSD. This is consistent with the model proposed by Pitman where an overactive amygdala would support the over-consolidation of the traumatic event. This hypersensitivity of the amygdala to general fearful stimuli (faces) was also found in a sample of individuals recently exposed to trauma (Armony, Corbo, Clement, & Brunet, 2005). In their study, the authors compared the activity of the amygdala to masked fearful versus neutral faces in subjects between four to six weeks after trauma. Their results indicate a significant correlation between symptom severity and the activity of the amygdala to the fearful faces, arguing further for the generally increased reactivity of the amygdala.

Following the data accumulated in the fear-conditioning field, researchers have also focused on the interaction between the amygdala and the ACC. As mentioned above, the model for PTSD combines both an exaggerated and over-consolidated fear acquisition with a chronic failure to learn and maintain extinction learning. This latter component led to studies looking at the co-activation of the amygdala and ACC in tasks that would demand suppression of an emotional response. One study by Shin and colleagues (Shin, et al., 1999) investigated the neural correlates of script-driven imagery of both neutral and trauma-related memories in groups of individuals suffering from childhood abuse-related PTSD and trauma-exposed victims of childhood abuse without PTSD. In reaction to the trauma-related scripts, individuals with PTSD showed less activation of the ACC compared to the trauma-exposed participants without PTSD. This

lack of activation of the ACC to traumatic memories was further replicated by Bremner and colleagues (J. D. Bremner, Staib, et al., 1999) in combat-related PTSD as well as with women suffering from childhood abuse-related PTSD (J. D. Bremner, Narayan, et al., 1999), victims of motor-vehicle accidents (Lanius, et al., 2001), and even adolescent victims of a natural disaster (Yang, Wu, Hsu, & Ker, 2004). Interestingly, when the symptom provocation scripts induced a significant dissociative experience, this reaction was not linked to a decrease but rather an increase of activity of the ACC (Lanius, et al., 2002). It may be that the decrease in ACC activation coupled with an increase in amygdala activity may underlie the preparedness to respond to a threat, whereas an increased ACC activity would lead to dissociation, i.e. the human equivalent of the freezing response in rats.

The decreased activity of the ACC in patients with PTSD was further investigated in a different paradigm where the subjects were not exposed to traumatic memories. This was performed using the Emotional Counting Stroop task (Whalen, et al., 1998) in a group of veterans from the Vietnam conflict. Subjects were asked to count the number of combat-related words displayed on a screen that were generally negative or neutral. Significantly less activity was observed in the rostral portion of the ACC when subjects counted more negative words. The authors interpreted the finding as a general failure of the ACC to suppress emotional reactions linked with the traumatic experience specifically.

Advances in neuroimaging studies have even allowed the investigation of the ACC and amygdala's co-activity through functional connectivity. Gilboa and colleagues (Gilboa, et al., 2004) published a report investigating the temporal relationship between amygdala and ACC reactivity to script-driven imagery using PET scans. When contrasting the trauma-related scripts with the neutral scripts, the authors observed an increase in amygdala activity and decrease in activity of the prefrontal region in patients suffering from PTSD. However, the efferents from the cingulate region projecting to the amygdala did not reach significance level in their analyses, which does not support the lack of inhibition of the ACC over the amygdala that is predicted by the fear-conditioning model of PTSD. On the other hand, Shin and colleagues (Shin, Orr, et al., 2004; Shin, et al., 2005) found that traumatized individuals with PTSD showed a significant negative correlation between the activity of the amygdala and ACC when shown either trauma-related or fearful faces. This finding also supports the model where PTSD includes the failure of extinction and habituation to fearful stimuli. Finally, additional support for the fear-conditioning model was found in a study from Felmingham and colleagues (Felmingham, et al., 2007) who examined the neural correlates of changes in symptom severity in patients suffering from PTSD. Specifically, the authors scanned individuals suffering from chronic PTSD following an acute trauma before and after cognitive restructuring therapy. They observed that, when subjects were shown fearful faces, the resulting level of activity of the rostral ACC post-treatment was positively correlated with the decrease in symptom severity, while the opposite pattern emerged for the amygdala.

Early studies on ACC in PTSD have investigated the integrity of the structure using anatomical brain imaging. The first report came from the team of Yamasue and colleagues (Yamasue, et al., 2003), who used the automated neuroimaging technique Voxel-Based Morphometry (VBM) to explore the grey matter density of individuals with a history of PTSD. Their results showed a decrease in density in the dorsal section of the ACC associated with PTSD. It must be noted however that out of all subjects in the clinical group, only one was currently suffering from PTSD while the others were all remitted. This limited the possibility to establish a clear link between PTSD and the structure of the ACC. The same year, Rauch and colleagues published the results from their study of the volume of the ACC in nurses with and without PTSD (Rauch, et al., 2003). Their results showed that the group suffering from PTSD had smaller rostral ACC volumes compared to the control group. This lends support to the fear-conditioning model. This was replicated in adults suffering from chronic PTSD following abuse during childhood (Kitayama, Quinn, & Bremner, 2006) and in veterans (Geuze, et al., 2008).

The portrait that emerges from all these studies is a confirmation of the fear-conditioning model. In PTSD an over-active amygdala would generate fearful reactions to trauma-related and sometimes unrelated but fear-relevant stimuli. Opposite to that, a hypo-active and potentially thinner ACC cannot provide the necessary inhibition over the amygdala further contributing to its overactivity. As well, a smaller hippocampus would be unable to fully process contextual

information and therefore unable to furnish the necessary input for successful extinction learning. However, as stated in the limitations of the fear-conditioning model, this focuses more on the first cluster of symptoms, flashbacks and intrusive memories. In terms of structural integrity of the brain, studies so far have not investigated other possible regions that may contribute to the other symptoms, namely peripheral hyperarousal, emotional numbing, social withdrawal and increased irritability.

Another important line of research in PTSD has focused on peripheral biological markers, notably the endocrine factors. The fear-conditioning model of PTSD underlies the importance of the stress hormones in the over-consolidation of the initial fear memory. The studies that were reviewed in the second section of this introduction have shown how glucocorticoids can modulate the consolidation and expression of fear in rodents and, to a certain extent, in humans. Based on this, studies have investigated the state of cortisol and the HPA-axis activity in individuals suffering from PTSD. We will review these studies in the following section.

4.5 Cortisol Levels in PTSD

The fear-conditioning model of PTSD proposes that the over-consolidation of the traumatic event depends on the increased release of stress hormones. As we have seen in the section on stress and fear-conditioning, glucocorticoids may modulate the strength of the memory. The interaction of glucocorticoids, specifically

corticosterone, and norepinephrine in the baslolateral amygdala has been shown to increase behavioral avoidance in contexts associated with an electrical shock. It is easy to draw a parallel between this behavioral response and the second cluster of PTSD symptoms, avoidance behavior, in human victims of trauma. Another argument for the hypothesized increased levels of cortisol in patients suffering from PTSD comes from the physiological correlates mentioned in the third cluster of symptoms, namely the hyperarousal and sleep-related issues. We have discussed in the section on stress how cortisol can affect multiple systems such as the cardiovascular system. One final argument for such a line of study comes from the memory alterations exhibited in PTSD, namely a partial recall of elements of the trauma and the often-observed lack of memory for neutral elements of the trauma. As some studies on verbal declarative memory in stressed humans have shown, cortisol seems to impair the recall of neutral elements and enhance recall of emotional ones, leading to a biased cognitive processing of the traumatic experience that would impair remission through extinction.

The field of endocrinology of PTSD has been very active in the past fifteen years. While a comprehensive review of this field is beyond the scope of this thesis, some critical aspects must be highlighted to understand the strengths and limitations of the findings gathered so far. First, as we mentioned earlier in the section on human studies of stress, the technique used to sample cortisol influences the conclusions that can be drawn from the studies. Many studies here have used either serum cortisol or urinary free cortisol, which are both

useful markers of the total quantity of cortisol secreted over a certain period of time. As we mentioned in the stress section above, both methods are limited in that they do not allow quantifying the pulsatile action of the HPA-axis. Fewer studies have used salivary cortisol, which allows the measurement of reactivity of cortisol as well as the CAR. Another aspect to keep in mind when reviewing studies on cortisol in PTSD is the actual population studied. The notion of chronic exposure to trauma versus time-specific trauma (e.g. trauma during childhood vs adulthood) may exert a different influence on the regulation of the HPA-axis. A third factor to keep in mind is the time since the trauma occurred. Studies on the effect of chronic stress have shown that the HPA-axis can modify its activity over time. The impact of unremitting symptoms on this system can therefore exert an influence that would not be related to the role of cortisol in the emergence of symptoms as it is hypothesized in the model.

One of the first reports on the levels of cortisol in victims of trauma was published by Resnick and colleagues (Resnick, Yehuda, Pitman, & Foy, 1995). In their study, the authors collected the plasma cortisol of victims of sexual assault within the first hours after the trauma and conducted a follow-up interview of the victims approximately 90 days after trauma exposure. Their results indicated that when they separated their subjects based on a history of sexual assault prior to the one that led to the emergency room, women with a history of assault showed lower levels of cortisol compared to women who were victims of a first assault. Furthermore, they observed that a history of assault was the best predictor of who might develop PTSD

at the clinical follow-up. Their results therefore contradict the proposed increase of cortisol in PTSD. In a further study, Resnick and colleagues did not replicate their first finding (Resnick, Yehuda, & Acierno, 1997): specifically, when they separated their subjects based on PTSD diagnosis, they observed that women with no prior history of assault who developed PTSD showed higher plasma cortisol in the emergency room compared to women with a history of prior assault who developed PTSD.

Since these original studies were published, conflicting data has also been reported. An early replication of the former findings by Resnick and colleagues came from the team of Kellner and colleagues (Kellner, Baker, & Yehuda, 1997) who showed that basal salivary cortisol in a sample of veterans from the Gulf War was negatively correlated with PTSD symptoms severity, arguing again for the blunted cortisol levels. This was further supported by data collected in victims of abuse (Heim, Ehlert, Hanker, & Hellhammer, 1998), mothers of child survivor from cancer (Glover & Poland, 2002), in women victims of early childhood sexual abuse (J. D. Bremner, Vythilingam, Anderson, et al., 2003), in motor vehicle accidents (Delahanty, Raimonde, & Spoonster, 2000), in treatment-seeking victims of a terrorist attack (Bierer, et al., 2006), various trauma types (Gill, Vythilingam, & Page, 2008) and war refugees (Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004). Interestingly, one study even showed how therapy could both improve symptoms and increase the basal levels of cortisol measured in plasma (Olff, de Vries, Guzelcan, Assies, & Gersons, 2007). One important aspect of this last study was that it was conducted with

patients suffering from PTSD following various trauma types. Considering the number of studies replicating the early findings of lower cortisol in PTSD, the conclusion of blunted levels of cortisol in patients with PTSD seems evident. However, the picture is not so clear.

Replicating the latter findings by Resnick and colleagues, Carrion and colleagues (Carrion, et al., 2002) found significantly higher levels of salivary cortisol sampled pre-lunch, pre-dinner and pre-bed, in pre-teenagers suffering from PTSD compared to healthy controls. Replicating this result, Weems and colleagues (Weems & Carrion, 2007) observed that the association between symptom severity and salivary cortisol levels at rest was positive only in children who had experienced a recent trauma, whereas in the distal trauma group, the correlation became negative. Interestingly, in a study of earthquake survivors, Song and colleagues (Song, Zhou, & Wang, 2008) found that increased serum over-night cortisol levels were present in all trauma-exposed subjects compared to healthy control participants, suggesting that an increase in cortisol was a marker of trauma-exposure and not PTSD. Laudenslager and colleagues (Laudenslager, et al., 2009) also published a report supporting the increased levels of cortisol in PTSD. A unique feature of their study was that the increased levels were present in both males and females, but females showed the increase later in the day whereas males showed the increase during the first hours of the day. This latter sexual dimorphism in cortisol abnormalities is important in view of the prevalent dimorphism in prevalence of PTSD as well as reactivity to fear-conditioning at large. Lastly, a recent

report by Steudte and colleagues (Steudte, et al., 2011) looked at cortisol present in the hair of victims from the war in Uganda with and without PTSD. In the group of individuals with PTSD, the authors observed a greater concentration of cortisol in the hair that corresponded to the last three months of life, compared with the trauma-exposed control group. This suggested a greater secretion of cortisol in this time-window, without specifying if that was a chronic and tonic condition or if surges of cortisol may have increased the levels present in the hair. In sum, there is considerable evidence of an increased level of cortisol in some victims of trauma.

Furthering the debate, a few studies have been published reporting no significant differences in cortisol levels between individuals with and without PTSD. In a study based on a wide sample from a community survey, Young and Breslau (Young & Breslau, 2004) failed to find a significant difference in urinary free cortisol between males and females who had a lifetime history of PTSD compared to trauma-exposed individuals who never developed PTSD. However, it must be noted that they did report a difference in women only, suggesting a sexual dimorphism in the cortisol levels. Golier and colleagues (J. A. Golier, Schmeidler, Legge, & Yehuda, 2006) also failed to report any significant differences in veterans from the Gulf War. This was further supported by another study from the same group (J. A. Golier, Schmeidler, Legge, & Yehuda, 2007). Interestingly, in these two studies, most subjects were males, replicating the absence of difference in males from the Young and Breslau study. However, this trend for an absence of findings in males only was contradicted by the

report of Metzger and colleagues (Metzger, et al., 2008) who also failed to show a significant difference in basal cortisol in female nurses with a history of PTSD compared to trauma-exposed nurses without a history of PTSD. Recently, van Zuiden and colleagues published the report of a prospective study following military personnel before and after deployment (van Zuiden, et al., 2010). They examined the cortisol levels in 318 soldiers before deployment and followed-up with a clinical assessment six months after their return. They observed that levels of cortisol did not predict the appearance of PTSD symptoms. These last studies indicate that the link between PTSD and abnormal cortisol levels is not yet clear.

4.7 Summary and conclusion from PTSD

In sum, the role of cortisol and the HPA-axis in PTSD is far from clear, since studies have been so varied in terms of techniques of sampling as well as populations studied. In order to better understand the potential role of cortisol in this disorder as it is suggested by the fear-conditioning model, we feel that studies should focus the investigation on samples drawn from the community, in adults exposed to a first acute traumatic experience. Conceptually, this sample presents the minimal conditions for the development of PTSD, since there is no chronic exposure involved and the trauma occurs when the HPA-axis is fully developed. Any differences observed can therefore be attributed to one specific event and not an interaction between the event and the natural development of the stress system. Additionally, there is a lack of studies that have used follow-ups and longitudinal

designs to illustrate potential changes in the HPA-axis due to the chronicity of the disorder or to the remission process. Lastly, considering the dynamic rhythm of the HPA-axis, there is a clear need to use a method that allows multiple sampling during the day. The results of Laudenslager and colleagues support this, as they found a sexual difference in the moment where cortisol abnormalities could be observed. Saliva samples and the measurement of the CAR are very useful tools to investigate the integrity of the HPA-axis that have been sparsely used so far.

Chapter 2: *Stress Reactivity, Personality and Fear-conditioning; a pilot project*

2.1 INTRODUCTION

For the past decades, studies have been conducted to try and understand the mechanisms by which individuals acquire fear. This emotion, which transcends cultures and even species, has been investigated using mainly associative learning based on Pavlovian conditioning. In its simplest form, this model predicts that, through enough pairings in time with an aversive stimulus (unconditional stimulus, US), a neutral stimulus can acquire the capacity to evoke lower levels of unconditional fear response (termed the conditioned response, CR) that typically follows the occurrence of the US. It thus becomes the conditional stimulus (CS+), contrasted with a similar stimulus that was never paired (CS-). This learning is not merely a temporal association, but the discovery of a significant predictive relationship between two stimuli. This learning is thought to be the result of increased activity within the amygdala, a small cluster of nuclei located in the anterior medial temporal lobe (J. E. LeDoux, 2000). The amygdala is densely connected with the hypothamalus as well as motor centers and the sympathetic nervous system (Critchley, 2002; Delgado, et al., 2006). This allows the measure, in humans, of the expression of fear through recordings of the galvanic skin response (GSR), i.e. the changes in electrical potential of the skin due to openings of the sweat pores. Many studies of healthy human subjects have shown that, independent of the exact nature of the CS and US, it was possible to observe increases of GSR that mirrored the activity of the amygdala (Cheng, et al., 2003; Knight, et al., 2004; Merz, et al., 2010; Stark, et al., 2006). Fear-conditioning is therefore an

interesting model to study fear and its potential role in the development of psychopathologies of the anxiety family, especially Post-Traumatic Stress Disorder (R. K. Pitman, 1989; R.K. Pitman, et al., 2001).

Few fear-conditioning studies conducted on human subjects have measured the endogenous levels of cortisol. This hormone is the end product of the hypothalamic-pituitary-adrenal axis (HPA-axis), which is widely considered the major stress axis of the endocrine system (Kudielka & Wust, 2010). In humans, this axis is known to respond to a variety of stimuli, both physical and psychological, by releasing corticotropin releasing hormone (CRH) from the hypothalamus, which binds to receptors in the pituitary gland, provoking in turn the release of adrenocorticotropic hormone (ACTH) in the blood stream. This will provoke the secretion of cortisol from the medullar cortex of the adrenal glands. Cortisol then feeds back to the central nervous system to modulate the activity of many key regions involved in fear conditioning, namely the anterior cingulate cortex, amygdala and hippocampus (Korte, 2001; de Quervain, Aerni, Schelling, & Roozendaal, 2009). The HPA-axis is known to be innervated by projections from the amygdala (Herman, et al., 2005; Rodrigues, et al., 2009). Furthermore, it responds not only to physical threats, but also to situations or stimuli that are novel and unpredictable, as well as threats to the ego only, through social evaluation (Dickerson & Kemeny, 2004). Considering that a fear-conditioning paradigm is a novel and unpredictable situation, it can be hypothesized that it would be enough to trigger the activation of the HPA-axis, which would lead to an

endogenous increase in cortisol levels. While physical threats are known to trigger the SNS, it is not clear if mild physical threats are sufficient to trigger a significant cortisol response. To date, only a few studies have investigated the link between fear-conditioning and endogenous cortisol to examine if fear is sufficient to induce a stress response. Further, previous studies have illustrated how secretion of cortisol can be modulated by factors such as personality traits (J.C. Pruessner, Hellhammer, & Kirschbaum, 1999), depression levels (M. Pruessner, Hellhammer, Pruessner, & Lupien, 2003) and early life parental care (Engert, Efanov, Dedovic, Dagher, & Pruessner, 2010). No study published so far has investigated the reactivity to a fear-conditioning paradigm, personality traits and reactivity of cortisol to the fear-conditioning paradigm, as well as the cortisol awakening response, which is known to be a good proxy measure of the integrity of the HPA-axis (Chida & Steptoe, 2009; Clow, Thorn, Evans, & Hucklebridge, 2004).

One study (Zorawski, et al., 2005) examined in healthy males and females the potential link between GSR and reactive endogenous cortisol. In their study, the fear-conditioning paradigm failed to elicit a significant cortisol response. However, when they performed a median split according to the cortisol levels 45 minutes post-task, they observed that males with higher cortisol responded more to the conditioned stimulus compared to the neutral stimulus. This effect was not present in females. However the authors failed to control for the stage of hormonal cycle in their female subjects or for the systematic use of oral contraceptives. It has been previously shown that female

gonadal hormones can affect levels of cortisol (Kirschbaum, et al., 1999; Viau, 2002) as well as reaction to a fear-conditioning paradigm (M. R. Milad, et al., 2006).

One important aspect of HPA-axis reactivity and cortisol secretion that has not been investigated in the context of human studies using fear-conditioning is the impact of early life experiences on both GSR reactivity and cortisol reactivity. Studies have shown that early life experiences modulate the expression of fear in context, but not cued, fear-conditioning in rodents (Bagot, et al., 2009), which is consistent with studies showing the influence of early life parental care on the development of the hippocampus and the stress axis (Zhang, et al., 2006; Buss, et al., 2007). In a recent study in our laboratory, it was observed that adults with low early life care showed an increased cortisol awakening response (CAR; Engert, Efanov, Dedovic, Dagher, et al., 2010). The CAR is a useful marker for the integrity of the HPA-axis (Kudielka & Wust, 2010). However, the effect of early life adversity on reactivity to a fear-conditioning paradigm in healthy adults has not been systematically studied.

The objective of the current study was to investigate the interaction of cortisol and GSR responses to a mild fear-conditioning procedure in young healthy adults. Specifically, we aimed at measuring the cortisol secretion pre- and post-acquisition of fear-conditioning in males and females (all on oral contraceptives). We also aimed at investigating how early life experiences and personality traits would modulate the acquisition of fear and cortisol reactivity to a fear-

conditioning paradigm. Last, we aimed at investigating the association between the CAR, personality and GSR reactivity.

We hypothesized that the fear-conditioning procedure would correlate with cortisol levels across our subjects. Further, we hypothesized a significant association between the CAR and the reactivity to the fear-conditioning task. Finally, based on Zorawski's findings, we hypothesized that there would be a significant sex effect on the response to the fear-conditioning task.

2.2 METHODS

2.2.1 Subjects and Procedure

Nineteen individuals (4 males) aged between 18 and 30 years old were recruited from classified ads. Potential subjects were excluded if they had a history of Axis-I disorder, a BMI greater than 27, were color blind or, for females, if they were not on oral contraceptives. Upon recruitment, subjects were invited for 2 visits separated by 72 hours, once for acquisition and then for extinction. Upon arrival, subjects were asked to sign the consent form. Following this, subjects were invited to rest and complete questionnaires for 60 minutes, before doing the acquisition task. During this time, subjects were asked to provide 4 saliva samples, once every 20 minutes. The conditioning task was done in a separate room where noise, light and temperature were kept constant. Once the acquisition task was completed, subjects were asked to rest for 40 minutes, during which 3 saliva samples were collected. Three days after acquisition, subjects were invited for a

second visit at the laboratory to complete the same procedure and perform the extinction task.

For exploratory analyses, only female subjects were analyzed. Since only one male qualified for the Responder group (see below), and considering the observed sex difference in GSR reactivity, inclusion of one male rendered statistical control for the influence of sex impossible. Therefore, only female subjects were included in the exploratory analyses.

2.2.2 Questionnaires

The Tridimensional Questionnaire (TPQ) was used to assess personality traits of Novelty Seeking, Harm Avoidance and Reward Dependence (C. R. Cloninger, Przybeck, & Svrakic, 1991). To assess for depressive symptoms, subjects were asked to fill the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, & Mock, 1961). Lastly, to measure the effect of early life adversity, subjects were asked to fill the Parental Bonding Index (PBI) for both care and overprotection.

2.2.3 Conditioning Task

The conditioning procedure was based on the task described in (Barrett & Armony, 2006). Specifically, the task was comprised of 4 blocks (CS+, CS+, CS-, Baseline) repeated 6 times throughout the task in randomized order. Each block was composed of presenting 10 cards

lasting 3 seconds, for a total of 30 seconds. During each card presentation, subjects were instructed to answer if two pictures, of either snakes or objects [chosen from the International Affective Picture System (IAPS)], were identical of different (see Figure 1 for a screenshot of the card). Subjects would submit their answer by clicking on the appropriate button located on the computer screen, using a computer mouse with their dominant hand. The CS was determined by the color of the background of the screen (CS+ = blue and green, CS- = orange, Baseline = purple). The task was presented on a 17-inch computer monitor located two feet away from the participants. The US was a burst of loud tone of 1.5KHz, generated with the freeware Audacity 1.2.5 and delivered through noise-canceling headphones (Sony MDR-NC7) at a volume set to each participant's tolerance of maximal volume (determined in a pre-testing session). When the US was paired, it would co-occur with one of the cards in a CS+ block. In total, 4 out of 6 blocks of CS+ were paired with the US. During extinction learning, subjects were re-exposed to the same procedure but no UCS was delivered. The conditioning and extinction task were programmed using the software SuperCard 4.5 (Solutions EtCetera Inc., USA).

2.2.4 Galvanic Skin Response Recording and Analysis

GSR was recorded using the BioPac MP 100 system (Harvard Apparatus Inc., USA), through LED electrodes applied on the skin of the index and middle fingers at the distal phalange on the non-dominant (left) hand (Cacioppo, Tassinary, & Berntson, 2000). Signal was

recorded for the whole task at a rate of acquisition of 200 Hz by the software Acknowledge 3.9.2 for MacIntosh computer (Harvard Apparatus Inc.). Using the text-format logfile produced by the conditioning program, each block of stimuli (CS+, CS- and baseline) was separated for analyses. Converting the graph file produced by Acknowledge into a text file (ASCII format), data was imported into the software MatLab 7.6 (MathWorks) for analyses. For each block, a baseline level was computed from the mean of the signal of the two seconds prior to the onset of the block. Subsequently, each block was divided into intervals of three seconds to mirror the onset/offset of the cards presented to subjects. For each card, a peak signal was recorded and subtracted from the baseline. All data was examined for the presence of outliers (mean ±3 s.d.) and transformed using the square root of the absolute value of the peak-baseline (Orr, et al., 2000). For statistical analyses, the first and last cards of each block were excluded, as they may have shown spill over effects from the previous blocks and on the following block. All statistical analyses were done using the SPSS 16.0 package for Macintosh Computers.

2.2.5 Cortisol samples and analyses

Saliva samples were collected using cotton salivettes (Sarstedt Co., Quebec City, Canada). Subjects were instructed to deposit the cotton swab inside their mouths without touching it with their fingers and keep the swab inside for 30-45 seconds. Samples were collected during testing, relative to arrival of subjects, at +0 minutes, +20 minutes, +40 minutes, +60 minutes (pre-task), +72 minutes (post-

task), +92 minutes and +112 minutes. For the daily samples, subjects were instructed to provide five samples: at awakening, 30 minutes post-awakening, 60 minutes post-awakening, at 4PM and 7PM, for two days. Cortisol levels were analyzed using fluorescenceimmuno assay (Kirschbaum, Strasburger, & Langkrar, 1993; Strasburger & Kohen, 1990). For diurnal cycles, the average of the two sampling days were computed for each subject. In order to increase compliance, all participants were given a written document with all appropriate information for the collection of samples at home. The document was then returned along with the samples for review.

For statistical analyses of cortisol, areas under the curve were computed according to the formula presented by Pruessner and colleagues (J. C. Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

3. RESULTS

Table 1 shows the socio-demographic characteristics of all subjects.

3.1 GSR and Cortisol at acquisition

A repeated measure analysis of variance across all subjects with Context (CS+/CS+/CS-) by Block (repetition 1 to 6) by Card (card no 2 to 9) as within subject factor revealed no difference between the CS+ and CS- blocks [F (2, 28) = 1.95, $p.> .16$] but a main effect of Card [F (7, 98) = 3.19, $p.< .005$]. Further analyses revealed that GSR

increased as a function of time (card no 2 to 9) inside each block, independent of the nature of the block (see Figure 2). A repeated measure analysis of variance also revealed a main effect of sample on cortisol levels [F (6, 108) = 3.69, $p.$< .002]. Specifically, cortisol levels during the task declined as a function of time (see Figure 3).

Correlational analyses across all subjects revealed a significant negative association across all subjects between AUC_g of the CAR and the reward-dependence sub-scale of the TPQ [r = -.448, $p.$ < .05; see Figure 4]. The AUC_i of the CAR was positively correlated with the harm-avoidance sub-scale of the TPQ across all subjects [r = .624, $p.$ < .004; see Figure 5]. The PBI was not significantly correlated with either AUC_g or AUC_i of the CAR (all $p.$ > .09). Further, a significant positive correlation was found between the subscale harm-avoidance and the saliva sample #7 [+40 minutes after the task; r = .531, $p.$ <.019; see Figure 6a], and a significant negative correlation between the reward-dependence subscale and saliva sample #7 [r = -.562, $p.$ < .012; see Figure 6b].

3.2 Exploratory Analyses

Using a *k*-means cluster analysis on the GSR to the US specifically, with female subjects only, we identified 2 subsets of individuals: responders (N = 5) and non-responders (N = 10). Using these subsets as groups, a mixed design analysis of variance with Group (responders vs non-responders) as a between factor and Context (CS+1, CS+2, CS-), Block (repetition 1-6) and Card (2 to 9) as

within factors revealed a significant interaction between Context and Cards [*F* (14, 182) = 2.02, *p.*< .008; see Figure 7]. Specifically, the later cards of the CS+ contexts showed greater GSR compared to the CS- context, independent of the Block repetition. Cortisol levels showed a trend towards a group difference across the testing session (*p.*<.07). When investigating cortisol levels pre-task (samples 1to 3), no significant interaction of group by sample could be detected [*F* (2, 24) = 1.54, *p.* < .24]. When examining the cortisol samples after the task (samples 5 to 7), again, no interaction could be detected [*F* (2, 24) = 2.60, *p.* < .10]. In both cases, there were no main effects of group.

3.3 GSR and Cortisol at extinction

At the second session 72 hours post-acquisition, three way analysis of variance with Group (responders vs non-responders) as a between factor and Context (CS+/CS+/CS-) and Block (repetition 1 to 6) as within factors revealed no significant differences in GSR for the interaction [*F* (10, 130) = 1.22, *p.*< .29] as well as for the Group by Context interaction [*F* (2, 26) = 0.56, *p.*< .58]. Similarly, a mixed-design analysis of variance with Group as between factor and Sample (1 to 7) revealed no differences in cortisol levels during the extinction task [*F* (6, 78) = 1.33, *p.*< .25]. Furthermore, independent-samples *t*-tests revealed no differences between Groups for CAR (all *p.*> .46) or on any subscale of the TPQ (all *p.*> .19).

4. DISCUSSION

In the current study, we examined the cortisol reactivity to a mild fear-conditioning procedure in healthy young adults without prior history of any mental health disorder. Our data revealed first that our fear-conditioning procedure was not effective across all our subjects. A number of factors might be responsible for this. First, the nature of the unconditional stimulus may not have been aversive enough to induce significant GSR. Most studies on fear-conditioning previously conducted have relied on electrical shocks. However, other studies have shown that a loud noise can induce significant conditioned GSR increases (Brignell & Curran, 2006; Jackson, et al., 2006), just as some studies have reported that an electrical shock may not be enough to induce significant conditioning (Stark, et al., 2006). Our *k*-means cluster analysis showed that only a third of our participants was reactive to this type of stimulus. It must be noted that our study design and analyses differed from the studies by Jackson and colleagues and Brignell and colleagues. One important factor was the contingency of pairings. Jackson and colleagues used a 100% contingency ratio and one CS+ unpaired to measure acquisition. Brignell and colleagues used a biphasic contingency ratio, with 100% for the early acquisition and 50% for late acquisition. In our study, we used a 0.60 ratio with the Context, and not Card, as CS, limiting the amount of US. It is possible that our absolute number of US may have been too low for the CS+ to become a good predictor of the US. Furthermore, Brignell and colleagues scored as 0 any GSR response that was smaller

than 0.01 μS, whereas we included all responses to fully capture the GSR reactivity. It may also be that loud noise by definition is not aversive enough for smaller samples of young healthy adults, who may be exposed in their daily lives to similar stimuli and thus habituate faster than older subjects. No study has so far compared the efficiency of various US to elicit strong conditioning, across different age groups. Interestingly, however, our fear-conditioning paradigm may have been aversive enough to induce acquisition in a smaller subset of individuals. Our data seems to indicate that explicit awareness of the CS-US association is not enough to trigger a significant GSR response to the CS+. Some studies have looked at the importance of awareness of the stimuli or the contingency. The data seems to indicate that awareness, while not necessary for the activity of the amygdala, seems to be necessary for the GSR expression (Tabbert, Stark, Kirsch, & Vaitl, 2006). It may therefore be that some individuals are more prone to pay attention and remain aware of the contingency. Since our CS was determined by the color of the background of the screen and not the pictures presented, some subjects may have paid more attention to the pictures and not the CS. An interesting avenue of studies would be to measure the exact visual attention dedicated to stimuli during a fear-conditioning paradigm. This may help us better quantify the saliency of the various CS used.

Another unexpected finding in our study is the reactivity of women to the fear-conditioning task. Previous studies (Zorawski, et al., 2005; Zorawski, et al., 2006; M. R. Milad, et al., 2006; (Stark, et al., 2006) have shown that women reacted less to a fear-conditioning

paradigm. However, other studies have shown a different pattern (Guimaraes, et al., 1991). Our data replicated the latter finding. One important difference between our study and those that have shown greater GSR in men is that all our female subjects were on oral contraceptives. Gonadal hormones have previously been shown to influence retention, but not acquisition, of fear-conditioning (M. R. Milad, et al., 2006). In the case of Milad and colleagues, they showed that men and women who were in the early phase of their cycle showed greater retention of extinction compared to women in mid-cycle. These authors did not investigate the effect of oral contraceptives on fear acquisition and retention. However, based on the levels of estrogen in the early phase and the effect of oral contraceptives on estrogen levels, it can be postulated that they would yield similar levels. Thus, we expected that the females in our study would not differ in GSR reactivity to the CS+ from the females studied by Milad and colleagues. Surprisingly, this was not the case. Based on the retention data from Milad and colleagues, we expected to see greater retention at the extinction phase in the female responders, which was again not the case. It may be that oral contraceptives influence other mechanisms involved in GSR, independently of their effect on estrogen. Systematic studies of levels of circulating levels of estrogen and other endocrine markers influenced by oral contraceptives and the menstrual cycle may yield a better picture of the actual factors responsible for the sex differences observed across studies.

Greater reaction to fear by women is, on the other hand, consistent with the epidemiological data from post-traumatic stress

disorder (Breslau, 2001), which is thought to be a fear-based disorder. The exact mechanism explaining the influence of gonadal hormones on fear-conditioning is not yet fully understood. This effect may be mediated by the influence of gonadal hormones on the HPA-axis as well as on vasopressin (Swaab, et al., 2005). Considering this influence of hormones on fear-conditioning, future studies looking at both fear-conditioning and PTSD would benefit from measuring the phase of the cycle as well as the use of oral contraceptives in female subjects.

Our study also revealed that a fear-conditioning paradigm, independent of reactivity of GSR, may not be sufficient to trigger a significant cortisol response, despite sharing elements of the typical stressor as identified by Dickerson and Kemeny (Dickerson & Kemeny, 2004). It also failed at provoking a significant increase in heart rate to the CS+. The cortisol finding replicates the global findings of Zorawski (Zorawski, et al., 2005). This may be again due to the nature of the unconditional stimulus, which may not be aversive enough to trigger a global response. It can also be due to the fact that, while a fear-conditioning procedure is unpleasant, it does not threaten the ego of the participants or their social selves. However, it must be noted that there was a trend in cortisol levels in our responders versus non-responders: responders seemed to show higher cortisol levels pre-task as well as at the sample 20 minutes after task. The *F* values for both tests were above 1, indicating a possible difference between groups. Our small number of subjects may have prevented us from detecting any small effect. Therefore, while this trend prevents us from concluding with great certainty that fear-conditioning is not sufficient

to elicit an HPA-axis response, it appears that greater number of subjects are necessary to detect such an effect. If this is the case, it raises the question of the strength of the potential influence of cortisol on fear-conditioning. At this point, we can only hypothesize what such an effect would be. Future studies could attempt to replicate our design with a mildly aversive stimulus to explore the characteristics of possible responders and non-responders to fear-conditioning, and more specifically the influence of the HPA-axis on these possible phenotypes.

Interestingly, we were the first study to examine the full CAR in young healthy adults exposed to a fear-conditioning paradigm. Mirroring the findings on the reactive cortisol during the task, the CAR was not significantly different between those who reacted and those who did not. The awakening response has been associated with self-esteem and early life adversity (Engert, Efanov, Dedovic, Dagher, et al., 2010). Studies have also shown that early life adversity may be associated with the development of key structures of the fear network such as the hippocampus. One study has shown that early life stress may impair fear conditioning in rodents (Kosten, Lee, & Kim, 2006). However, no such studies have been conducted in humans. Our results do not indicate any link between parental bonding and GSR reactivity, though this may be due to our small sample size. Considering the emerging literature on early life adversity and stress reactivity, the addition of a GSR component could increase our knowledge of the development of the psychophysiology of emotion.

Our analyses further revealed a significant positive correlation between AUC_i of the CAR and the harm-avoidance subscale of the TPQ. In other words, greater increase of cortisol in response to awakening was associated with greater harm avoidance. Interestingly, the CAR was not associated in our samples with the PBI, which reflects the level of parental care during early life experiences. According to Cloninger's initial biosocial personality theory (C.R. Cloninger, 1986), harm avoidance subscale would be associated with the levels of serotonin. Interestingly, some disorders have been shown to display abnormal levels of cortisol and to respond to medication targeting serotonin. For example, depressive patients have been shown to exhibit greater harm-avoidance (Quilty, Godfrey, Kennedy, & Bagby, 2010), a trait that would mediate the effect of clomipramine. A similar picture appears when we look at Post-Traumatic Stress Disorder. One study has shown that the CAR assessed with salivary cotisol showed that subjects suffering from PTSD had a blunted CAR (Wessa, Rohleder, Kirschbaum, & Flor, 2006) and that a normal CAR would be a resilience factor (see chapter 4). Similarly to depression, individuals suffering from PTSD were found to display higher harm-avoidance relative to the normative data (Richman & Frueh, 1997). One study even found that harm-avoidance assessed before exposure to trauma helped predict who was at greater risk of developing symptoms upon exposure (Gil, 2005). In their study, the authors had screened over 185 students for personality. Out of them, 81 were exposed to the same traumatic event and were called back by the investigators. Their results showed that, while trauma exposure did not change personality traits, harm-avoidance was strongly associated with symptoms severity assessed

six months after the traumatic event. Therefore, the picture that emerges from both these disorders is a possible combination between blunted CAR and higher harm-avoidance. Our sample did not show such an association, independent of whether they responded to the fear-conditioning task or not. However, correlations highlighted a positive association between harm-avoidance and cortisol levels post-acquisition, specifically the sample at 40 minutes post-acquisition, which replicates the sample used by Zorawski and colleagues to differentiate their high/low cortisol groups (Zorawski, et al., 2005). The timing of this sample may be interpreted as the return to baseline level. This correlation may therefore represent that individuals with high harm-avoidance do not return to baseline as quickly as individuals with lower harm-avoidance. However, this interpretation is limited by the fact that our task did not induce significant increase in cortisol levels.

This study suffered from some limitations. First, our fear-conditioning paradigm was not successful in inducing a significant acquisition across all subjects. As mentioned above, many factors may have influenced the effectiveness of the paradigm, such as the number and nature of the US, the saliency of the CS+ and the inclusion of all GSR, independently of their level. The lack of a clear inter-trial interval may have also led to a habituation in the succession of CSs, diminishing the startle response to the occurrence of the CS. Based on the work of Rescorla (Rescorla, 1988b), we propose that the two main factors explaining our failure to induce significantly greater GSR in response to the CS+ was the low contingency rate as well as the weakness of the

US. Additionally, it appears that our relative small number of participants, based on previous studies, limited our capacity to detect an effect. This was highlighted by the trends observed for cortisol levels. For this pilot study, we recruited only 4 males compared to 15 females, which limited our capacity to detect significant sex differences in acquisition and retention of conditioning. Lastly, while most studies examine retention of conditioning with a 24 hours delay, we used a 72 hours delay. It is possible that mild fear-conditioned responses are forgotten after such a period of time, preventing us from detecting any significant retention.

In sum, our data seem to indicate that simple fear-conditioning may not be sufficient to trigger a full HPA-axis response. It may be that the stress axis is activated only in response to specifically social situations where demands exceed the resources or in cases of more serious threats to the integrity of the person, in support of the SNS. Additionally, it is possible that the HPA-axis reacts only to the most severe situations. Considering its wide ranging effects and high costs in terms of resources, it is possible that safe-guard mechanisms allow for only SNS activation without concomitant HPA response if the threat does not warrant the full defensive response. This being said, if fear-conditioning is to be used as a model for the acquisition and consolidation of post-traumatic stress disorder, then future studies would benefit from including a modulation of the stress hormones, as it seems to be involved in this pathology. Animal models have already indicated that increases in pre-training cortisol enhance the consolidation of fear-memories, especially but not exclusively for

context-based conditioning (Rodrigues, et al., 2009). Human studies with a focus on better quantifying the interaction between sympathetic nervous system, HPA-axis, sex and personality could potentially better grasp the reality of traumatic exposure across individuals. This may help better predict who is at greater risk for developing PTSD upon exposure and thus lessen the burden by preemptively targeting interventions where and when they are most needed.

Figure 1. Screenshot of conditioning task window

Pictures displayed for 3 seconds.

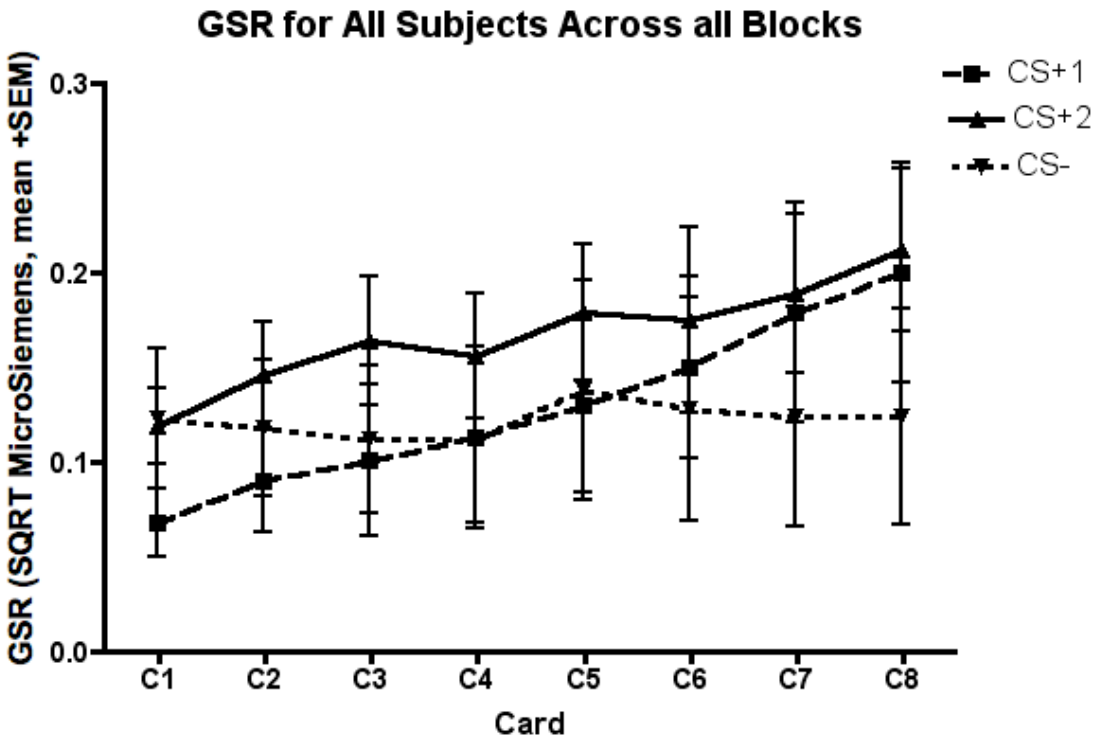


Figure 2. GSR at Acquisition across all subjects

No significant main effect of Stimulus across all subjects (*p.*> .16), indicating that subjects failed to respond more to the CS+ (1 or 2) compared to the CS-, independent of the repetition (1 to 6) of the stimuli.

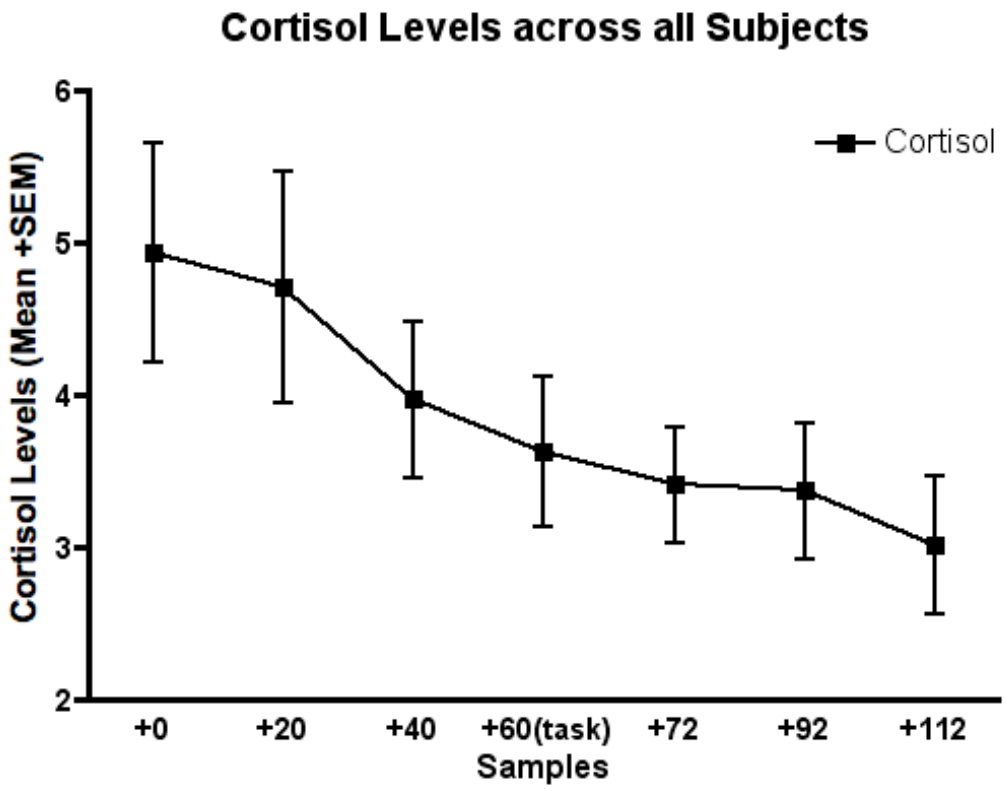


Figure 3. Cortisol levels during Acquisition

Main effect of time on Cortisol levels: significant decrease of cortisol levels throughout the testing period across all subjects, indicating that the fear-conditioning task did not induce a cortisol response (cortisol levels in nmol/L).

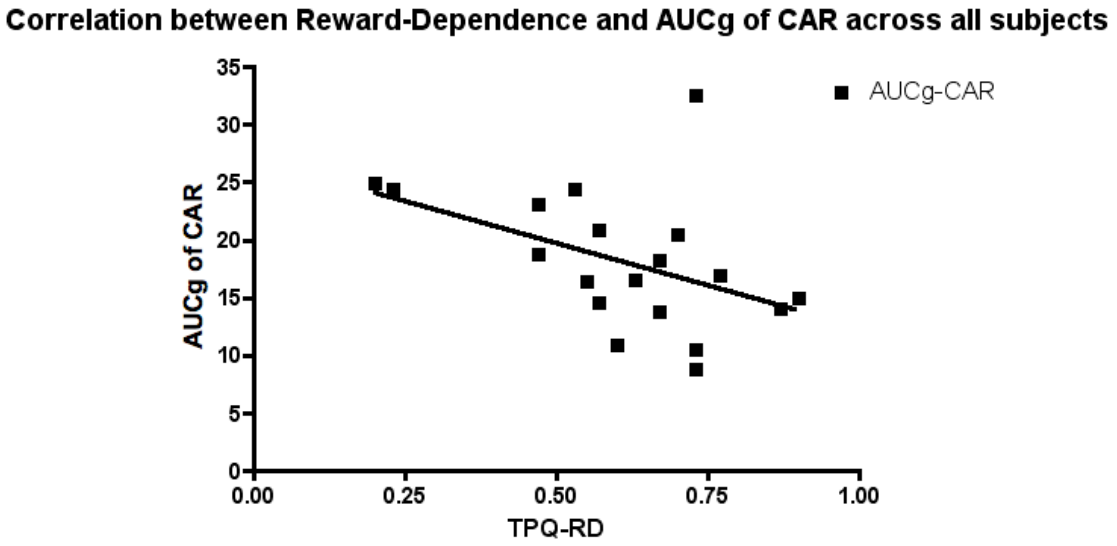


Figure 4. Correlation AUC_g of CAR and Reward Dependence Correlation between Reward Dependence assessed with the Tridimensional Personality Questionnaire and AUC_g of CAR, *r.* = -.448, *p.*< .05

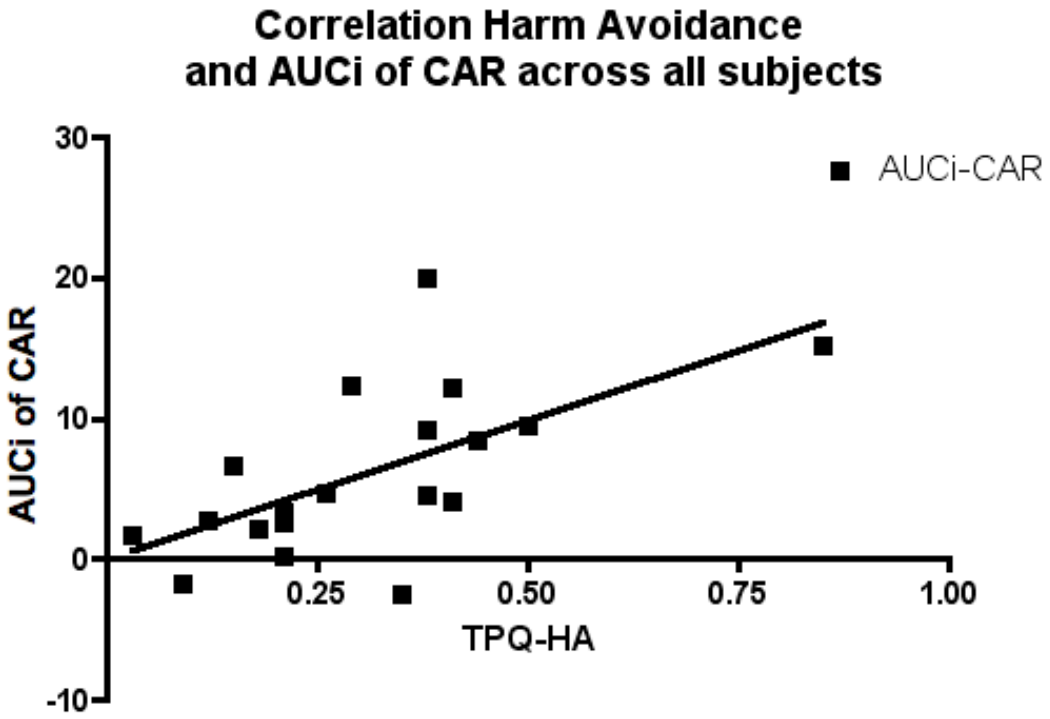


Figure 5. Correlation AUC_i of CAR and Harm Avoidance

Correlation between Harm Avoidance assessed with the Tridimensional Personality Questionnaire and the AUC_i of the CAR across all subjects, *r.* = .624, *p.*< .004.

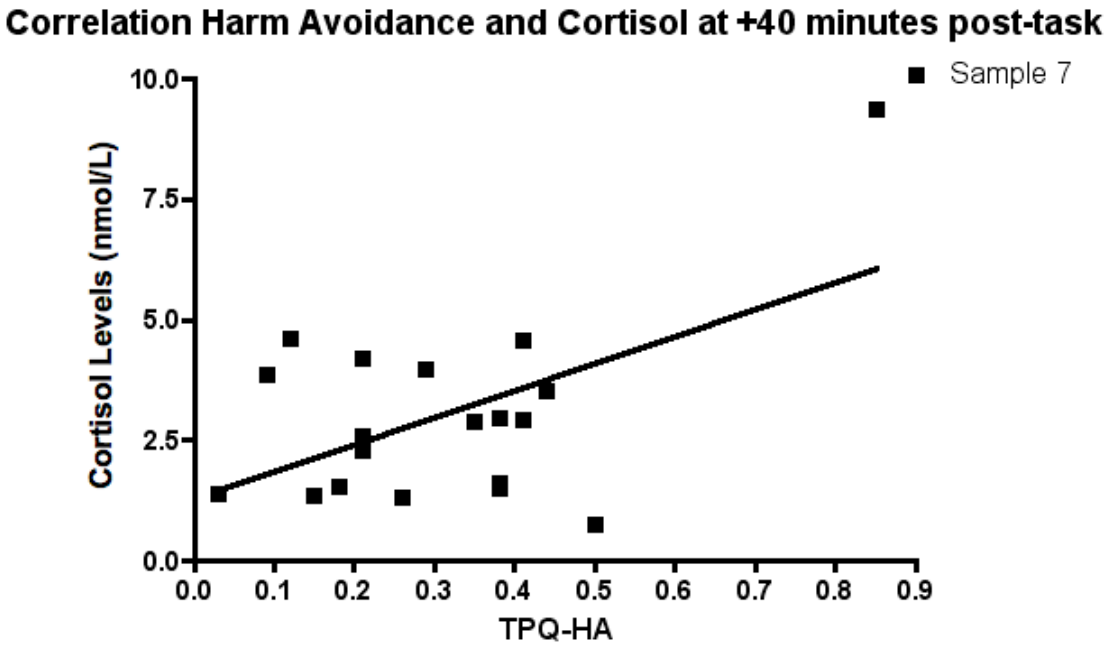


Figure 6a. Correlation Cortisol post-task and Harm Avoidance

Correlation between Harm Avoidance assessed with the Tridimensional Personality Questionnaire and Cortisol levels at sample 7 (40 minutes after acquisition task), $r. = .531$, $p. < .019$

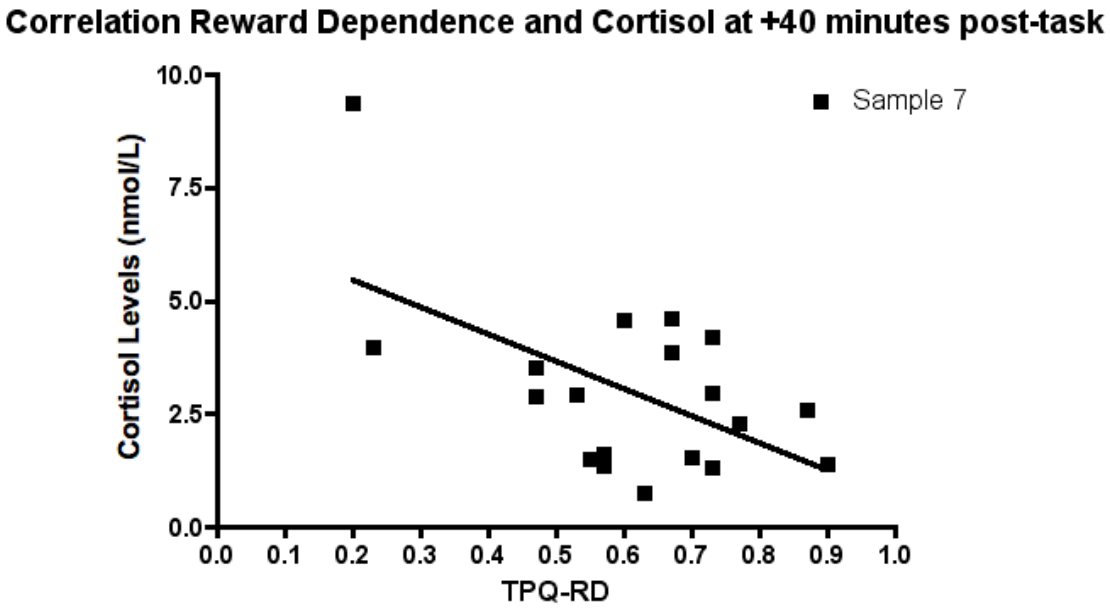


Figure 6b. Correlation Cortisol post-task and Reward Dependence Correlation between Reward Dependence assessed with the Tridimensional Personality Questionnaire and Cortisol levels at sample 7 (40 minutes after acquisition task), *r.* = -.562, *p.* < .012

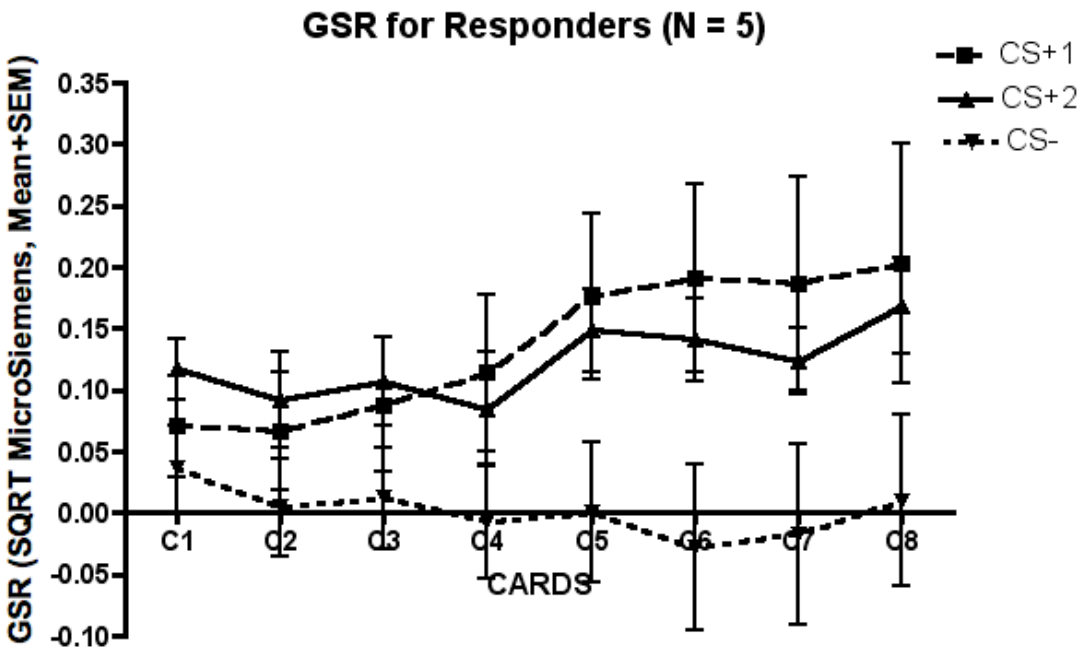


Figure 7. GSR Reactivity in Responders

Responders according to a *k*-means cluster analysis showed significantly greater GSR response at the end of the block (cards 5-8) for both CS+ compared to CS-, independent of the repetition during the task (blocks 1 to 6).

	All	Responders	Non-Responders	*p.*
Age	22.81 (.93)	23.60 (2.29)	23.22 (1.46)	.89
Sex (M:F)	4:15	0:5	0:10	.29
BDI	5.39 (1.30)	4.40 (2.29)	5.13 (2.22)	.83
TPQ-NS	.55 (.03)	.60 (.06)	.54 (.05)	.13
TPQ-HA	.31(.04)	.25 (.06)	.32 (.04)	.31
TPQ-RD	.61(.04)	.69 (.05)	.61 (.04)	.40

Table 1: Socio-demographic data

Showing mean (standard error of the means); BDI = Beck Depression Inventory; TPQ = Tridimensional Personality Questionnaire; NS = Novelty Seeking; HA = Harm Avoidance; RD = Reward Dependence; Note that for the Responders/Non-responders classification, males were excluded since they were not sufficiently represented in each group.

Chapter 3: *Fear-Conditioning, Social Stress and Extinction Learning in healthy adults*

3.1 INTRODUCTION

Based on the findings from our previous study showing that fear-conditioning did not induce significant reactivity of the HPA-axis measured with cortisol, we now turned to a second study investigating the effect of post-acquisition cortisol levels manipulations on immediate extinction. As mentioned earlier, fear is a basic emotion that is considered to be the root cause of some psychiatric disorders, from simple phobias to post-traumatic stress disorder (PTSD) (APA, 2004). The latter is described as a failure of a victim of trauma to regain a sense of safety once the traumatic situation is over. Hence, the individual suffering from PTSD may re-experience elements of his trauma and may display abnormal levels of fear to neutral stimuli that became associated to the trauma (Brunello, et al., 2001). In order to better understand these symptoms, studies have used the model of Pavlovian fear-conditioning to examine the conditions under which one can learn to fear or not to fear specific stimuli (R. K. Pitman, 1989).

This model describes how a previously neutral stimulus paired with a naturally aversive or threatening stimulus (termed unconditioned stimulus, or US) can eventually elicit a fearful response (conditioned response, or CR) even in the absence of the US (J. E. LeDoux, 2000; Rodrigues, et al., 2009). It thus becomes a conditioned stimulus (CS+). Unpaired neutral stimuli (CS-) however will not provoke this CR. In the context of PTSD, a victim of assault may display fearful reactions to an item of clothing that the aggressor wore during the assault, such as a green baseball cap (CS+). The learning of fear is

thought to be mediated at the neurological level by the amygdala, a bilateral cluster of nuclei that are located in the medial temporal lobes (Cahill, et al., 1996; Cahill, Weinberger, Roozendaal, & McGaugh, 1999; LaBar, et al., 1998; J. E. LeDoux, 2000; Phelps, et al., 2004). This structure possesses important connections to the sympathetic nervous system (SNS) that allows the measure of fearful reactions through levels of the electrodermal activity, or more commonly of the Galvanic Skin Response [GSR; Critchley, 2002]. Therefore, individuals that show greater increase of GSR to the CS+ compared to the CS- are thought to have acquired fear to the conditioned stimulus. Lastly, just as someone can learn to fear a neutral stimulus, it is possible to inhibit the display of the CR when enough CS+ have been presented without any occurrence of the US, a process termed extinction learning. This process thus describes the CR decrease in time down to a level that is comparable to pre-conditioning levels.

As we mentioned in chapter 1, the current model used to study PTSD (R. K. Pitman, 1989; R.K. Pitman, et al., 2001), based on fear-conditioning, posits that the traumatic event is an overly intense fear-conditioning situation that provokes the massive activity of the sympathetic nervous system through the important release of catecholamines, especially norepinephrine. Another aspect of the model is the hypothesized activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis in response to the traumatic event, through connections from the central nucleus of the amygdala to the hypothalamus (Rodrigues, et al., 2009). This neuroendocrine axis is responsible for the release of the hormone cortisol, which is

considered the main stress hormone in humans (Swaab, et al., 2005; Kudielka & Wust, 2010). The HPA-axis has been shown to modulate learning and memory in humans, increasing verbal declarative memory of emotional stimuli (Cahill & McGaugh, 1998; Maheu, et al., 2004). However, few studies have investigated the interaction between endogenous cortisol and fear-conditioning, using the activity of the SNS as the main outcome measure instead of verbal declarative memory. One study has reported that the use of exogenous hydrocortisone in young females increased the GSR to the CS+ vs CS- at acquisition, and it also increased reactivity to the CS- relative to the CS+ during extinction (Tabbert, et al., 2010). However, another study reported that cortisol had an effect during acquisition only in male subjects (Stark, et al., 2006). In the case of clinical use for anxiety disorders, a review by DeQuervain and colleagues (de Quervain, et al., 2009) has shown how glucocorticoids can impair the retrieval of the emotional memories and thus diminish symptoms of PTSD or phobia. The question therefore remains if endogenous cortisol may enhance the strength of the CR or if it may impair its retrieval in healthy humans.

In a combination of two studies, Zorawski and colleagues (Zorawski, et al., 2005; Zorawski, et al., 2006) investigated this question by exposing their participants to a fear-conditioning paradigm using pictures of spiders and snakes as CS and a brief electrical shock as US. Following this procedure, their subjects from the second study underwent a psychosocial stress task that used mental arithmetic to induce activity of the HPA-axis. The following day, participants came

back to the laboratory to undergo extinction learning. Using a median-split to separate subjects in high and low cortisol levels (independent of the stress or control condition), the authors observed that the males in the high cortisol group displayed greater response to the CS+ at the late acquisition stage but not at the 24h retention test. This study therefore suggested that variation in endogenous cortisol did not systematically modify the reactivity to the CS+ across all subjects. However, it did suggest that late acquisition cortisol correlated to differential response to the CS+ only in males at acquisition (pre-stress). Lastly, it did not confirm the potentiating effect of cortisol on consolidation of memory. Our first study replicated some of the findings of the first study by Zorawski and colleagues, namely that cortisol levels did not seem to increase as a reaction to a simple fear-conditioning paradigm. This finding supported the use of additional methods to manipulate the activity of the HPA-axis in a fear-conditioning paradigm.

An important factor to note from the previous set of studies from Zorawski and colleagues is that they failed to consider three important elements in regards to the HPA-axis reactivity. First, the HPA-axis reactivity to a stress task must be taken into context of the global capacity of the axis to produce cortisol. One avenue that allows indexing the integrity of the HPA-axis is the measurement of the Cortisol Awakening Response (CAR), which represents the sharp increase of cortisol that occurs in the first 30 minutes following awakening (Kudielka & Wust, 2010; Linkowski, et al., 1993; Schmidt-Reinwald, et al., 1999). Further, the CAR and general reactivity of the

HPA-axis have been shown to be modulated by early life experience (Engert, Efanov, Dedovic, Dagher, et al., 2010), a factor that may be related to the integrity of structures of the central nervous system that modulate the activity of the stress response (Buss, et al., 2007). A third and important modulator of the HPA reactivity, as well as GSR activity, are the gonadal hormones (Kirschbaum, et al., 1999; Kajantie & Phillips, 2006; M. R. Milad, et al., 2006). It has been shown that females at various phases of the hormonal cycle display varying degrees of GSR and endocrine responses to the same challenge, highlighting the importance of controlling for this factor.

Considering the previously stated factors, the aim of this study was threefold. First, we wanted to investigate the effect of an acute psychological stressor, post fear acquisition, on immediate extinction in young healthy men and women. Second, we examined the association between GSR reactivity to a mild fear-conditioning task and the integrity of the HPA-axis, using the CAR as a predictor of GSR reactivity. Third, we examined how retrospective assessments of early life care and adversity would influence both the GSR and cortisol reactivity. We hypothesized that individuals exposed to a social stress will show greater extinction learning. Further, we hypothesized that there will be a significant negative association between the CAR and GSR reactivity. Last, we hypothesized that childhood adversity will be significantly associated with GSR and cortisol levels during the task.

3.2 METHODS

3.2.1 Subjects and Procedure

Forty participants (20 males) were recruited from classified ads. All subjects were from 18 to 30 years old, non-smokers, and screened for history or current Axis-I disorder, history of trauma exposure, and history of Axis-I disorders in first-degree relatives. Potential subjects were excluded if they were colorblind, had a BMI greater than 27 and, for females, if they were not on oral contraceptives, in order to control for cortisol levels.

All testing was conducted in the afternoon. Figure 8 shows the detailed protocol. Specifically, upon arrival, subjects were given questionnaires to fill out during 40 minutes. At this time, they were escorted from the resting room to the testing room, where the instructions for the fear-conditioning task were given. Once the fear-conditioning task was completed, sixty minutes after arrival, subjects of the Stress group were given the instruction for the social stress task while going from the conditioning room to the stress room. There, subjects were introduced to the panel and given 5 minutes to prepare for the task. Following this, subjects underwent the social stress task, which lasted ten minutes. The subjects of the control group were given time to read emotionally neutral magazines in the resting room for the same duration. After the social stress task, subjects went immediately back to the conditioning room where they underwent the extinction paradigm. Once this was completed, subjects went back to the resting room, where they were given twenty minutes to complete

the questionnaires or to read the emotionally neutral magazines. This completed the testing session. Saliva samples were collected, relative to time of arrival, at +30 minutes, +45 (pre-acquisition), +60 (post-acquisition), +65 minutes (anticipation of stress), +70 minutes (pre-social stress), +80 (post-social stress), +85 (pre-extinction), +100 (post-extinction), +110 and +120.

3.2.2 Questionnaires

Depressive symptoms were assessed using the Beck Depression Inventory. Early life adversity was measured by the Parental Bonding Index as well as the Childhood Trauma Questionnaire. Both trait and state anxiety were measured using the Spielberger Trait and State Anxiety Index. Finally, dissociative trait was assessed using the Dissociative Experience Scale version 2.

3.2.3 Conditioning Task

Considering the low (30%) rate of acquisition in our previous sample, a different paradigm was selected for this study in an attempt to increase rate of acquisition across all subjects. The fear-conditioning task was therefore based on the task validated by Brignell et al. (Brignell & Curran, 2006), which also used noise as US in healthy adults, and programmed using the software SuperCard 4.5 (SolutionsEtcetera Inc.). Based on our previous paradigm and its low success rate, we changed key elements of the paradigm in order to increase conditioning. Specifically, the task was divided into two

phases, contrasting with the single phase of the first paradigm. The first phase was the habituation, where subjects were presented with 4 CS+ without US and 4 CS-. Following this phase, a set of instructions were displayed on the screen for 30 seconds, reminding the subjects that the next phase would be the actual conditioning task. The conditioning phase began with 4 presentations of the CS- and 4 of the CS+ paired with the US, for a 100% contingency rate (compared with a total of 6 presentations of the US in the previous task). This was meant to increase the contingency between CS and US and therefore the predictive value of the CS. The order of the presentation was randomly selected by the program, but it was insured that no more than 2 CS+ would be presented together. Following this, subjects were exposed to 16 CS-, 8 CS+ paired with the US and 8 CS+ unpaired. These last stimuli were the ones used for analyses. The order of the presentation was again randomly selected with the insurance that no more than 2 CS+ would be presented in a row. The nature of the CS was again determined by the color of the background of the screen (grey for CS-, red for CS+) and lasted 3 seconds, followed by an inter-trial duration of 12 seconds. This change from a longer (30 seconds) to shorter stimulus (3 seconds), with greater ITI (12 seconds in the current task versus 0 in the previous task), was chosen to increase general startle as well as reactivity to the CS as an event and increase the capacity of the CS (versus all other stimuli) at predicting a possible US. The US was again a loud noise of four different frequencies (between 1200Hz and 1500Hz) generated with the freeware Audacity 1.2.5. It was delivered through noise-canceling headphones (Sony MDR-NC7). The volume of the US was adjusted

according to each subject's tolerance, which was assessed prior to the task. Subjects were instructed to make sure the noise was as loud as they could tolerate without it being painful. The onset of the US occurred 2 seconds after the onset of CS+ and would co-terminate with the CS+.

During the task, subjects were instructed to indicate if two shown pictures were identical or different and answer by clicking on the appropriate button on the screen. The extinction paradigm was a replication of the acquisition, though no US was delivered at any time. Subjects were unaware that the second phase presented no US and were still asked to wear the headphones.

3.2.4 Social Stress Task: TSST

The Trier Social Stress Task (Kirschbaum, Pirke, et al., 1993) is a validated tool to induce an endogenous cortisol increase in response to a purely psychological stressor. For this task, subjects were instructed that they would have to perform a mock job interview in front of a panel of experts in behavioral observation. Further, they were told that their performance would be recorded by a camera for further examination and review. Subjects were then introduced to the panelists, who were instructed to remain completely neutral and give no emotional feedback, either negative or positive. They were then given time to prepare their job interview by taking notes, though they were instructed that they would not be able to bring the notes with them in the testing room. The first part of the TSST is the mock job

interview that lasts for five minutes. Following this, participants were asked to perform a mental arithmetic task, such as subtracting 17 from 2023 serially until they reach 0. If they made a mistake, a panelist told them that their answer was incorrect and that they should start over from the beginning. After five minutes, subjects were told that the panelists had enough information and that they could step out of the testing room. This concluded the task itself. At the end of the testing session, subjects were debriefed by the experimenter and told that the task is meant to make people uncomfortable and the panelists are instructed not to give any feedback.

3.2.5 Galvanic Skin Response and Heart Rate Recording

All recordings were done using the BioPac MP 100 system (Harvard Apparatus, USA). Electrodes filled with specially prepared gel were applied to the first phalange of the index and middle fingers of the non-dominant hand (Cacioppo, et al., 2000). Subjects were instructed to keep their hands as still as possible to avoid movement-induced artifacts. Electrodes for heart rate measurement were applied at the level of the hips on both sides as well as a grounding electrode one inch above the anklebone on the left leg. This insured reliable and non-invasive recording of heart rate. Recordings were done at 200Hz for the whole duration of the task, both for acquisition and extinction.

3.2.6 GSR Analyses

From the raw recordings done with the BioPac software Acqknowledge 3.9.2, files were converted into ASCII text files. Based on the logfile produced by the conditioning task, markers were added to the basic test file to indicate the onset of each stimulus by type (CS-, CS+ paired, CS+ unpaired). Based on the protocol for GSR analysis validated by Orr (Orr, et al., 2000), we identified the baseline level by finding the mean level of GSL for the two seconds prior to the onset of the CS. For each stimulus, the duration of the event (3 seconds) and the following inter-trial interval (12 seconds) were divided into three intervals of 5 seconds each. For each interval, the peak value was identified and subtracted from the baseline value. This corresponded to the GSR. All values were then inspected for outliers and square root transformed to normalize the distribution. For statistical analyses, the average value of the CS+ 1-2, 3-4, 5-6 and 7-8 were used and contrasted with the average values of CS- 5-8, 9-12, 13-16 and 17-20 to reflect the early, mid-early, mid-late and late acquisition (Quarters 1 to 4). Analyses were done using the software MatLab 7.6 (Mathworks Inc.) and SPSS 11.0 for MacIntosh computers.

3.2.7 Heart Rate Analyses

Using the raw recording from the BioPac software Acknowledge 3.9.2, recording for the acquisition phase was isolated using the crop tools of the software. Based on the sequence of stimuli from the logfile generated by SuperCard, separate sub-files were generated for

each condition (CS+, CS-, US). Using the specialized scripts from Acknowledge, heartbeats were classified for each condition and mean heart rate was extracted.

3.2.8 Cortisol Sampling and Analyses

Cortisol samples were collected using salivettes (Sarstedt Co., Quebec City, Canada). Specifically, subjects were instructed to slide the cotton swab inside their mouth while avoiding contact with the fingers, and to keep the swab in their mouth for at least 30 seconds. Once this time elapsed, subjects were instructed to again slide the swab back in the tube without touching it with their hands. Once samples were completed, they were stored in -20 Celsius freezers. All samples were analyzed using fluorescenceimmuno assays (Kirschbaum, Strasburger, et al., 1993; Strasburger & Kohen, 1990).

3.2.9 Statistical Analyses

Three way analyses of variance (ANOVA) were used with Quarter (1 to 4) by Stimulus (CS+ vs CS-) by Group (Stress vs Control) as between group factors, controlling for sex, and GSR as the dependent variable. Three way analyses of variance (ANOVA) were used with Phase (Acquisition vs. Extinction) by Stimulus (CS+ vs. CS-) by Group (Stress vs Control), with sex as covariate, were used to analyze Heart Rate. Cortisol values were analyzed using a mixed-design analysis of variance with Sample as a within-subject factor and Group as a between-subject factor. Also, the area under the curve (AUC) with

respect to ground (AUC_g) and with respect to increase (AUC_i) were computed for the CAR according to the formula validated by Pruessner et al. (J. C. Pruessner, et al., 2003). These values were correlated using Pearson's correlations with the scores obtained on the personality questionnaires. All alpha values for the correlation analyses were corrected for multiple comparisons using a Bonferroni's correction.

4. RESULTS

4.1 Acquisition of Fear-Conditioning

Between group comparisons revealed no differences of age, level of education, depressive symptoms, trait and state anxiety and cortisol levels pre-task (all *p.*> .05, see table 2). A mixed-design repeated measure ANOVA with Stimuli (CS+ vs CS-) and Time (Q1-Q4) as within-subject factors and Sex as covariate revealed no significant effect of stimuli (CS+ vs CS-) on the GSR across all subjects [*F* (3, 114) = 1.03, *p.*< .09]. Further, there was no interaction between sex and stimuli on the GSR, nor any effect of sex on GSR [*F* (1, 38) = .337, *p.*< .55].

Using a *k*-means cluster analysis based on the GSR to the US throughout acquisition, specifying 2 means, groups were established with Responders (n = 16; 6 males, 10 females) and Non-Responders (n= 24; 14 males, 10 females). Groups of responders did not differ in terms of state or trait anxiety or depressive symptoms (all *p.*> .05). A repeated measure mixed-design ANOVA with Group (Responders vs

Non-reponders) as between-subject factor and Time of CS+ as within-subject factor revealed a significant effect on GSR [F (3, 114) = 2.87, $p.$ < .04; see figure 9]. *Post-hoc* analyses revealed significant differences at Quarter 3 and 4.

4.2 Cortisol levels and Stress Task (TSST)

When selecting only the Responders, we used a mixed design ANOVA with Group (TSST vs Control) as a between factor and Saliva Sample as a within factor to investigate the effect of the stress task on cortisol levels, controlling for sex. This revealed a significant Group by Sample Interaction [F (4, 52) = 4.73, $p.$< .000, see figure 10], where the TSST group showed increased cortisol levels compared to the Control group only at Samples 7, 8 and 9, corresponding to 5 and 20 minutes post-TSST (i.e. pre and post-extinction), and 10 minutes post-extinction. When selecting all subjects exposed to the TSST, independent of reactivity to the fear-conditioning acquisition, we compared the responders and non-responders to the MOCT. A mixed-design ANOVA with Group (Responders vs Non-Responders) as between subject factor and Samples as within-subject factor revealed no significant difference of reactivity to the TSST between responders and non-responders to the fear-conditioning paradigm [$p.$ >.80].

After computing the area under the curve of cortisol secretion due to the TSST, both with respect to the ground and to the increase, correlations were done between AUC_g and AUC_i and the trait and state anxiety scores acquired before acquisition, across all subjects of the

TSST group. Significant correlations were found between AUC_g and trait anxiety [r = .523, $p.$< .03] as well as between AUC_i and trait anxiety [r = .561, $p.$< .019] (see figure 11a). No correlations were found for state anxiety (see figure 11b).

4.3 Extinction of Fear-Conditioning

Looking at the reactivity to the CS+ in the extinction phase in Responders only, a mixed-design ANOVA with Time (Q1-Q4) as within-subject factor and Group (TSST vs Control) as a between subject factor revealed a significant interaction between factors [F (3, 30) = 3.54, $p.$ < .03]: *post-hoc* analyses revealed that the mean GSR of the TSST group was significantly smaller than that of the Control group at Quarter 3 (see figure 12).

4.4 Heart Rate Analyses

A three way analysis of variance revealed no significant interaction between Phase, Stimulus and Group [F (1, 30) = .388, $p.$ <.54]. A significant Phase by Group interaction was detected [F (1, 30) = 6.72, $p.$< .02]; *post-hoc* analyses revealed that while heart rate decreased in the Control group between Acquisition and Extinction, heart rate did not differ between phases in the Stress group (see Figure 13). Further, there was a significant main effect of Sex [F (1, 30) = 4.62, $p.$< .04]: *post-hoc* analyses revealed that females showed higher mean heart rate compared to males, independent of Phase, Group or Stimuli.

When selecting only the Responders, a significant interaction between Phase, Stimuli and Group was observed [F (1, 11) = 5.04, $p.$< .05]. *Post-hoc* analyses revealed that, for the CS+ only, mean heart rate differed between Groups at the extinction phase only (Figure 14). No differences were observed for the CS-.

4.5 Childhood Adversity and Personality

Investigating the effects of childhood trauma on personality traits, we performed correlations between the Emotional Abuse and Physical Abuse sub-scale of the CTQ and depressive symptoms, dissociation experience and trait anxiety. Analyses revealed strong positive correlations between Emotional Abuse and depressive scores [r = .607, $p.$< .000; see figure 15], as well as dissociative experience [r = .667, $p.$< .000; see figure 16].

4.6 Childhood Adversity and Biological Markers

Correlations were also performed on the average reaction to the CS+ across acquisition for all subjects and childhood emotional abuse and physical abuse. No significant relationships were found between these factors [all $p.$> .05 post Bonferroni].

When investigating the association between emotional abuse, physical abuse and the CAR, no significant correlations were detected with either AUC_g or AUC_i across all subjects. When splitting subjects

according to sex, we detected marginally significant correlations between AUC_g of the CAR and emotional abuse [*r.* = -.535, *p.* <.04] and physical abuse [*r.* = -.559, *p.* <.02] in females only (not Bonferroni corrected, see figure 17A and B). No association was detected in males or with AUC_i of the CAR.

5. DISCUSSION

In this study, we aimed at assessing the impact of endogenous rise of cortisol on immediate extinction of a cue-based fear-conditioning task in young healthy men and women. Our first analysis showed that our fear-conditioning paradigm failed to elicit significant reactivity to the CS+ across all our subjects. Through a *k*-means cluster analysis of the reactivity of participants to the US, we identified two groups of Responders (n = 16) and Non-Responders (n = 24) that subsequently differed in terms of reactivity to the CS+, based on their GSR. Subjects of the Stress group (n = 20) were exposed to a social stress task immediately following the acquisition stage, which induced a significant increase in the stress hormone cortisol, compared to the subjects of the Control condition (n = 20), and independently of the Responder to fear-conditioning status. Immediately after the stress task, all subjects were re-exposed to the fear-conditioning paradigm in an extinction-learning task. Our data showed that, among the Responders, those who had been exposed to the stress task showed faster decline in the GSR to the CS+ compared to Responders of the control condition.

Following the acquisition phase, half of our subjects were exposed to a social stress task, the Trier Social Stress Task. We replicated the previously reported efficiency of the TSST to induce significant cortisol increase. Interestingly, the increase was found in all subjects exposed to it, independent of whether they responded to the fear-conditioning acquisition. This finding is interesting in that it underscores a possible independence of reactivity to fear and stress in laboratory setting. It also shows that prior triggering of the SNS does not induce a greater or smaller stress response. Other studies have illustrated the effect of pre-acquisition exogenous cortisol on fear-conditioning (Merz, et al., 2010; Stark, et al., 2006; Tabbert, et al., 2010), showing the potentiating effect of cortisol on the activity of the amygdala that has been previously observed in rodents (Rodrigues, et al., 2009). However, this is the first study to use an endogenous cortisol increase to modulate immediate extinction learning. The crucial finding that our study revealed was a faster rate of extinction in the responders exposed to stress compared to the control condition. This raises the question of the exact role of cortisol in emotional learning and extinction. While some studies mentioned previously have indicated a potentiating role of cortisol on the consolidation of the CR and even on emotional verbal memory, our findings suggest that cortisol might possess a time-dependent effect that may modulate learning itself. Cortisol administered at different stages may not have the same effect on the CR. Pre-acquisition exposure may increase consolidation of the CR, while pre-extinction may hinder the retrieval or expression of the CR, leading to more efficient extinction. This finding echoes some data obtained in the field of PTSD as well as other anxiety

disorders, where cortisol has been proposed to be used as an enhancer for psychotherapy (Yehuda, 2009; Yehuda & Golier, 2009). Our conclusions are limited by the small number of subjects exposed to the stress conditiong, indicating a need to replicate our design in larger samples. Furthermore, we cannot at this stage conclude to an effect of cortisol *per se*, as it may simply be the performance of another task that may have interfered with the fear-conditioning. Future studies should investigate through pharmacological treatment the effect of cortisol increases, using hydrocortisone, or a social stress task without cortisol increases, using metyrapone, on acquisition of extinction. Despite the limitations of our study, our results indicate that the field of psychoneuroendocrinology of fear-conditioning and stress requires more investigation to better characterize the interaction of fear and stress in humans. Another interesting factor that our data showed is the correlation between the total amount of cortisol released as a consequence of the TSST and the trait anxiety as measured by the Spielberger's anxiety scale. It is important to note that trait anxiety in our study correlated with stress and not fear. This might help further understand the exact interaction between the constructs of fear, stress and anxiety, which are all involved in PTSD.

Our data raise some interesting questions. First, only 40% of our participants could be conditioned to fear using our protocol. Interestingly, the study by Stark et al. (Stark, et al., 2006) also revealed the presence of responders and non-responders to a different paradigm. While these authors reported rates of response around 40-45% across all their subjects, we obtained a similar success

rate. This mirrors recent findings from the animal literature (D. E. Bush, Sotres-Bayon, & LeDoux, 2007). These authors reported phenotypical differences in acquisition of fear and extinction in genotypically identical rats, arguing for a natural occurring variability in acquisition. However, the low rate of reactivity in our study may be due to a number of factors relating to our paradigm. The most obvious factor may be the actual nature of the US used, mirroring our first study. In our studies, the US was a burst of loud noise of high frequency, with the volume adjusted to each participant's individual tolerance. This type of US has been used successfully in previous studies (Barrett & Armony, 2006; Brignell & Curran, 2006; Jackson, et al., 2006). Most studies so far have used electrical shocks as US, though not always successfully (Stark, et al., 2006). Since no study has systematically compared the efficiency of both stimuli in creating associative learning, we cannot comment on the respective capacity of each stimulus with complete certainty. A burst of loud noise may not be as aversive as an electrical shock to young healthy adults, since most young adults are used to the presence of a loud noise in daily urban life. The result of this is that loud noises may startle at first but subjects may habituate fast to their presence, leading to a steady decline in GSR.

Beyond the nature of the US, another possible factor that may have contributed to our task not eliciting greater conditioning across all our subjects could be the CS itself. First, contrasting with our previous study described in Chapter 2, the CS was of a short duration (3 seconds), which may not have been long enough to allow a full GSR.

The duration was selected, based on the protocol of Brignell and colleagues (Brignell & Curran, 2006), to minimize the habituation and increase startle. Also, the difference between CS+ and CS- was based on the color of the screen. While colors have been used before in studies as CS [e.g. (Barrett & Armony, 2006; Jovanovic, et al., 2006; M. R. Milad, et al., 2006], it may be that the simple contrast between red and grey might not be salient enough for individuals to learn and display significant discrimination between the two CSs. Instead, the simple onset and offset of a CS would become the salient event and therefore would provoke an increase in electrodermal activity, independent of the nature of the event.

Most studies of fear conditioning have used a habituation phase for subjects to be exposed to the CS without any occurrence of the US. However, this phase may provoke an inhibition of CS-US association acquisition through latent inhibition (Maren, 2001; Rescorla, 1988b), especially if the US is only mildly aversive. Also, we must note that many studies [e.g. Alvarez, et al., 2008; Blechert, et al., 2007; Jackson, et al., 2006; Kalisch, et al., 2006; Knight, Nguyen, & Bandettini, 2006; M. R. Milad, et al., 2006; Moratti, et al., 2006; Tabbert, et al., 2010] use a paradigm where the acquisition phase shows a high rate of CS-US pairing, sometimes close to 100%. In our study design, based on the paradigm of Brignell and colleagues (Brignell & Curran, 2006), we used a biphasic schedule of pairing, starting at 100% for the first four occurrences of the CS+, followed by a 50% pairing rate for the following 8 CS+. This schedule may not have been enough to induce the CS-US association, since the CS+ predicted the

US only 50% of the time past the first 4 CS+. This was in proportion lower to the contingency of pairings from our first study, although the absolute number of US delivery was higher.

Finally, one aspect of our statistical analyses that must be mentioned is that we analyzed the data from all CS+ displayed during the acquisition phase. Some studies have set criteria for including or excluding a GSR in their analyses, or converting them to a value of 0 (even if the actual GSR was of a negative value). Furthermore, for example, after pairing the CS+ with the US at a 1:1 ratio, Jackson and colleagues (Jackson, et al., 2006) presented only one or a few CS+ alone to assess acquisition. In our study, among the Responders, we saw significant increases in reactivity to the CS+ only at the later stage of the acquisition phase. This inclusion of more GSR data points may have influenced our statistical analyses. It may also help better represent acquisition in a time-dependent fashion, an aspect that is not represented in many studies.

One interesting factor about our Responders group that goes against the current literature is the higher number of females compared to males. Most studies that have assessed fear-conditioning in humans have generally reported greater GSR in males compared to females (Jackson, et al., 2006; Zorawski, et al., 2006; Zorawski, et al., 2005), albeit not always (Guimaraes, et al., 1991). Interestingly, however, is the fact that fear-conditioning is a model for disorders such as Post-traumatic Stress Disorder, which is known to affect more women than men (Breslau, 2001). This discrepancy between the

model and the actual disorder raises the fundamental question as to the factors that may increase the prevalence of fearful responses and the chronic failure of extinction in females specifically. One hypothesis rests on the impact of gonadal hormones in females, which have been shown to influence the acquisition and retention of fear-conditioning (M. R. Milad, et al., 2006). Our study design included only females that were on oral contraceptives, which are known to modulate the levels of circulating estrogen. Considering the effect of estrogen on cortisol secretion and the stress response (Fries, Dettenborn, & Kirschbaum, 2009; Kirschbaum, et al., 1999), it must be noted that all studies investigating fear should include measures of the menstrual cycle or systematic screening for oral contraceptives, in order to control for the effect of estrogen on biological markers such as GSR in their female subjects.

Our analyses revealed an interesting pattern of correlations between emotional and physical abuse and the global amount of cortisol secreted in response to awakening in female subjects only. This association did not translate into a greater increase in the morning or to males. This might indicate a sex effect of early life adversity on the programming of the HPA-axis. Furthermore, childhood adversity did not correlate with GSR. This seems to indicate that the development of reactivity of the fear network and stress axis may not be in synchrony, but rather possess different critical windows. One study from our team highlighted the importance of maternal care in mediating the impact of pre-natal stress on the development of the hippocampus only in females (Buss, et al., 2007). This echoes the

findings from animal studies on the epigenetic influences on HPA-axis development (Bagot, et al., 2009; Meaney, 2001). It has been shown how the hippocampus helps regulate the activity of the HPA-axis (Dedovic, et al., 2010). Our results support the effect of childhood experiences mostly in females, but not in males, which raises the question of the factors that may possess a similar influence in males.

Childhood emotional and physical abuse, despite not being correlated with general markers of biological reactivity, was strongly correlated with two other factors, namely depressive scores and dissociative experiences. It is interesting to note that both factors are also related to PTSD. One of the most common comorbid conditions that is found in individuals suffering from PTSD is major depression (Brunello, et al., 2001). As for dissociation, it is a common feature of the peri-traumatic experience as well as a symptom of the disorder (APA, 2004; J.D. Bremner, et al., 1992; Marmar, et al., 1994). In our sample, it appears that a developmental pattern emerges in which childhood adversity may increase the vulnerability of individuals in case they face traumatic experiences by increasing the occurrence of dissociation. But since the adversity was not severe, it is possible that behavioral patterns emerged without a significant biological counterpart, representing a risk factor in case of later trauma exposure.

Coming back to fear-conditioning as a model for PTSD and the traumatic exposure, our project was innovative in highlighting the differential effect of cortisol post-acquisition on immediate extinction.

If we combine the effects of our data with the results reported by Zorawski and colleagues (Zorawski, et al., 2006), it would appear that cortisol has a time-specific effect, depending on when the exposure to the CS+ is occurring. If impacting early post acquisition, while cortisol levels are at their peak, it would seem that cortisol might potentiate a decrease in the GSR. However, if memories are left to engage in consolidation, cortisol may increase the consolidation of the CS-US association and therefore augment the reactivity to the CS+ alone at the long-term memory stage, only in those subjects who present high levels of cortisol. This could result in greater difficulty for extinction learning, even though the data from Zorawski and colleagues do not support fully this conclusion. In sum, the human data does not replicates the findings from the animal literature (Rodrigues, et al., 2009), where glucocorticoids have been shown to influence long-term memory assessed with percentage freezing to the CS+, both in cue-based and context-based conditioning, but not short-term memory. Our study instead points towards an effect on short-term memory and subsequent extinction learning. Future studies in human subjects should investigate the effects of post-training endogenous cortisol on both short- and long-term memory in larger samples and with multiple assessments. This may be a better way to model the peri-traumatic experience, as individuals exposed to trauma may be presented with reminders of the event (CS+) at an acute stage, whether in the form of interviews by police officers, paramedics or doctors. Furthermore, future studies should investigate the link between verbal declarative memories and GSR reactivity, as both seem to be differently modulated by cortisol. Finally, it must be noted that fear-conditioning

paradigms conducted in laboratory settings are mildly fearful. Even though they do elicit reactivity of the SNS and central nervous system structures such as the amygdala, hippocampus and anterior cingulate cortex, the low levels of reactivity suggest that fear-conditioning may be a model for basic learning of fear; it is questionable if this model is really appropriate to describe the actual peri-traumatic experience. The limitations imposed by the laboratory setting may prevent us from achieving a model that fully represents the abnormal levels of arousal and emotional distress of the traumatic experience. New studies may be needed to explore other dimensions and possible modifications of the current model.

The hallmark of Pavlovian fear-conditioning is the capacity to elicit a CR to any CS, as artificial as a simple color or a Rorschach image. But investigating PTSD and the peri-traumatic reactions may require future studies to go beyond pavlovian fear-conditioning towards a mix of context and cue-based conditioning where virtual reality may be used to increase the realism of the conditioning context, and where glucocorticoids are assessed and modulated post-training in order to better mimic the natural sequence of biological events. The use of more natural or customized stimuli may increase the realism of the conditioning paradigm and allow for a better assessment of the factors involve in the acquisition, extinction or maintenance of fear, factors that may be generalized better to real-life settings. This line of investigation may help explain better the cascade of events that happens at the time of trauma, in the

immediate and crucial aftermath, as well as the long-term effects of such a disorder as post-traumatic stress disorder.

In sum, our study provided interesting findings on the interaction between cortisol and GSR. While most animal studies have shown an increase in fearful reactions due to cortisol, our study shows the opposite pattern. This must be interpreted as a safeguard when translating findings from rodents to humans. Cortisol appears to modulate verbal memory and SNS activity differently. As both systems are involved in the reaction to trauma, this argues for a more comprehensive model of PTSD that would integrate both aspects, as well as the developmental challenges that may skew the activity of both HPA-axis and SNS towards greater reactivity. Such a model is necessary if we are to better predict who may not remit from a traumatic event.

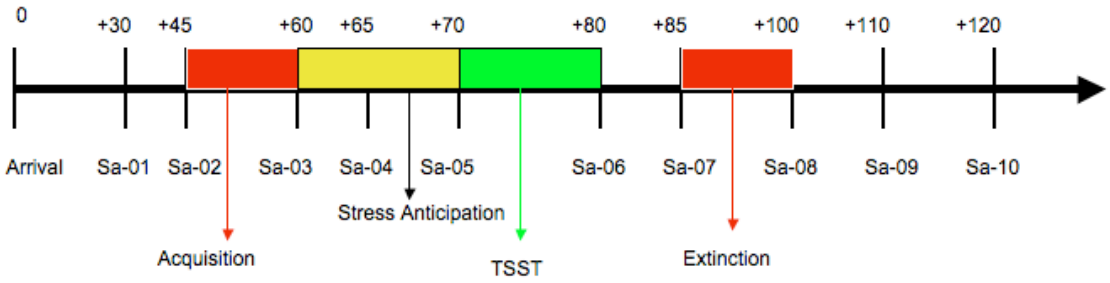


Figure 8. Experimental Design

Description of the procedure. Arrival corresponds to time 0. Each vertical bar represents minutes after arrival. Yellow rectangle corresponds to the anticipation period for the TSST in the Stress group only; subjects of the Control condition read magazines. 'Sa' corresponds to saliva samples. The testing session terminated at +120 minutes after arrival.

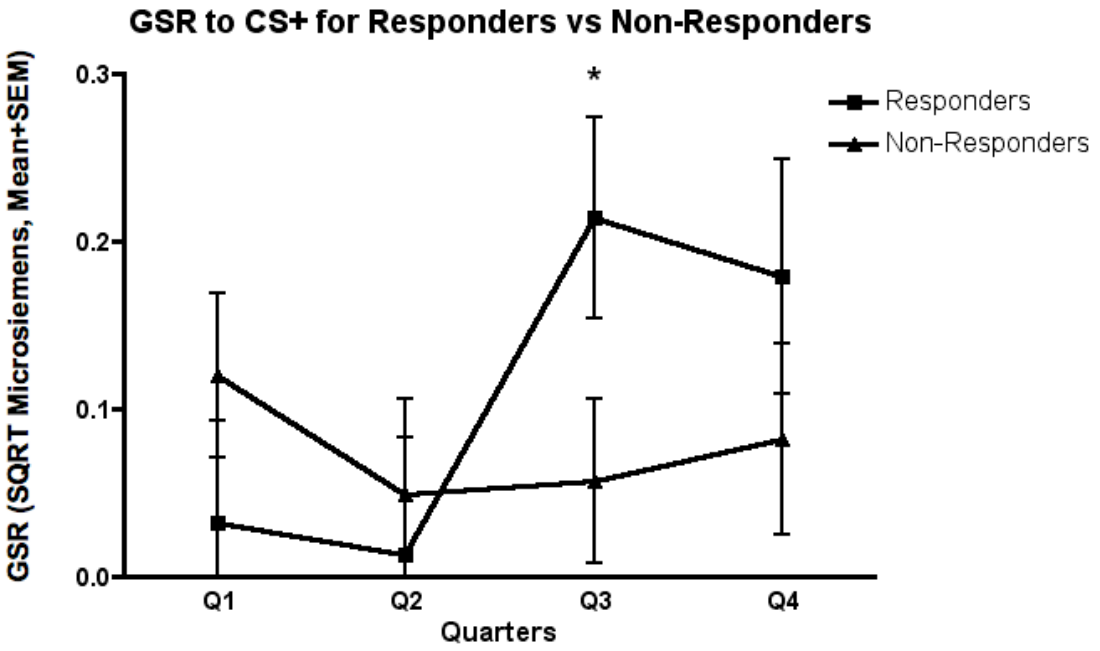


Figure 9. GSR to CS+ at Acquisition for Responders/Non-Responders Significant Quarter by Stimulus interaction [F (3, 114) = 2.87, $p.$ < .04] indicates that conditioning occurred at Q3-Q4. FIR corresponds to the first interval response, from onset of task card to 5 seconds. All data represent mean, error bars represent standard error of the mean.

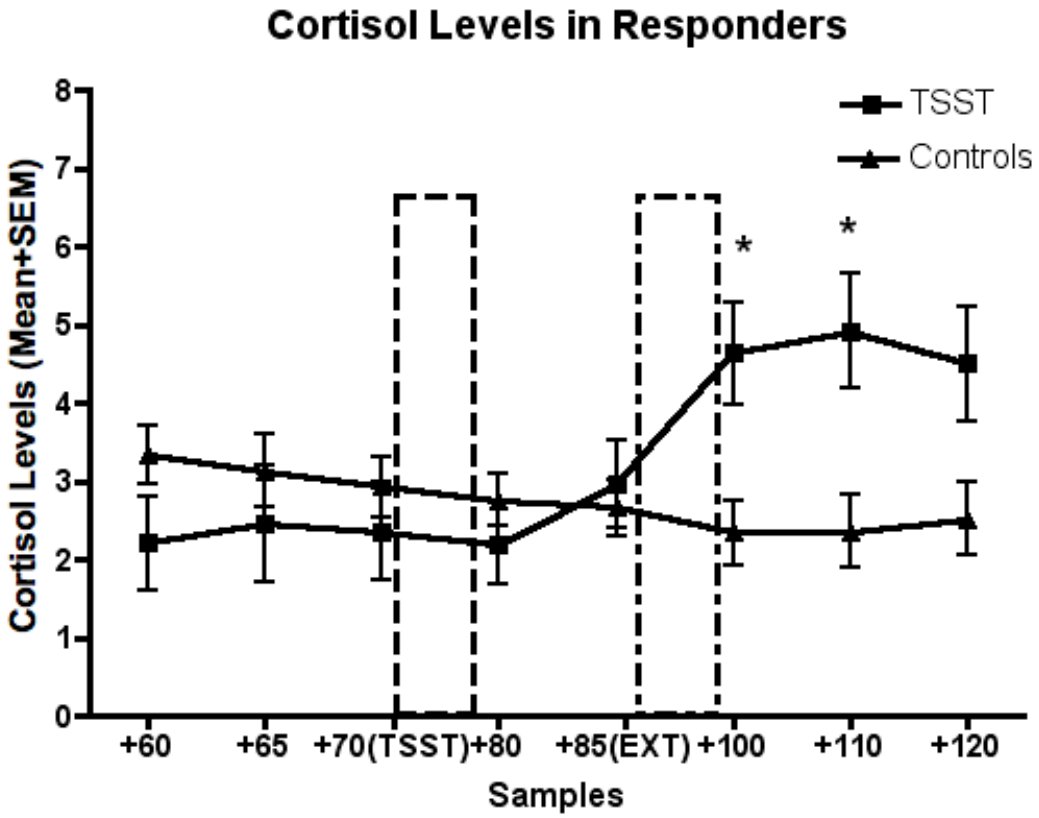
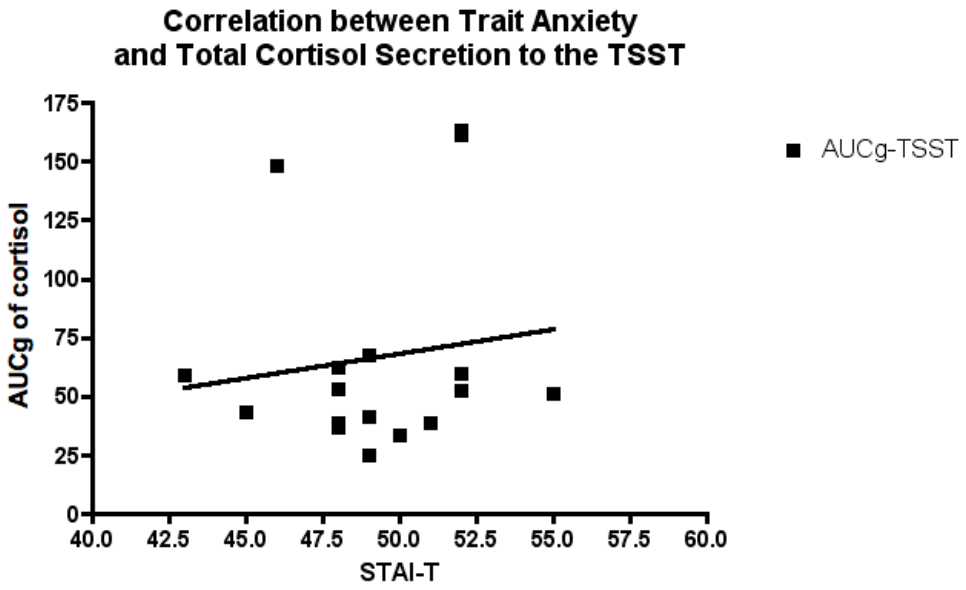


Figure 10. Cortisol levels at TSST and Extinction in Responders

Cortisol levels controlling for sex in Responders to the acquisition phase only. The left (dashed) rectangle represents the timing of the TSST; the right rectangle represents the timing of the Extinction phase. Significant differences in cortisol levels are present at the +100 and +110 minutes relative to arrival. Cortisol levels are in nMol/L. All values are means and error bars represent standard error of the mean.

(A)



(B)

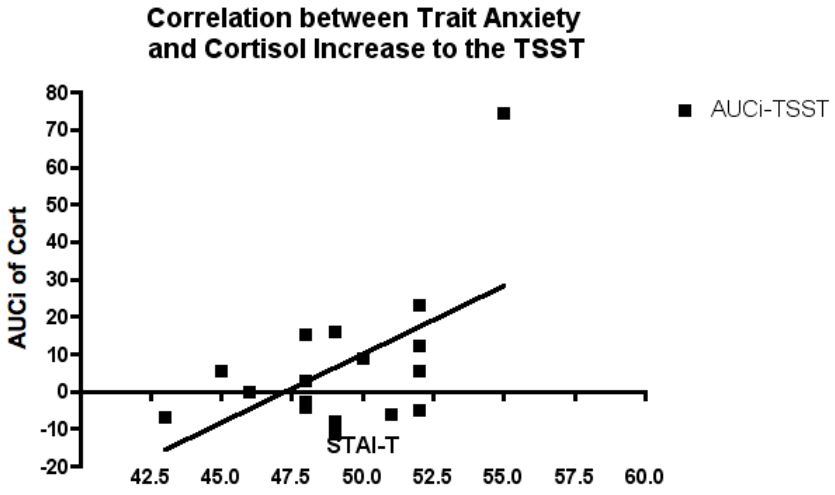


Figure 11. Correlations between Anxiety cortisol in the TSST

(A) Correlation between AUC_g and trait anxiety r^2 = 0.27; (B) Correlation between AUC_i and trait anxiety r^2 = 0.31.

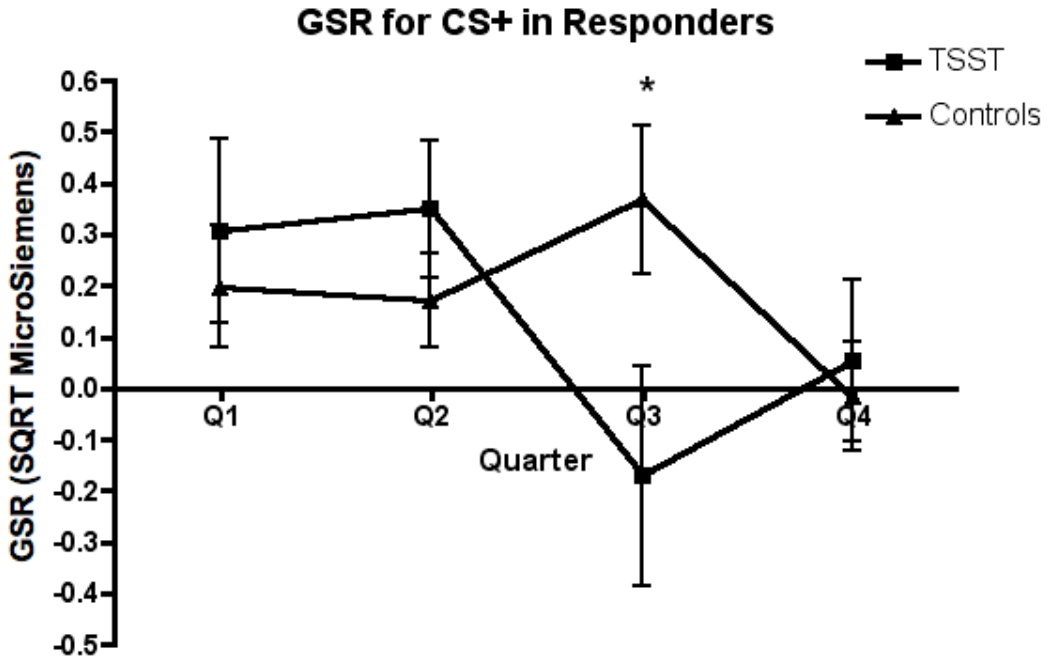


Figure 12. GSR to CS+ at Extinction in Responders

Shown is the reactivity to the CS+ in Responders at Extinction. The group exposed to social stress shows a significant decline at Q3 compared to the Control group [F (3, 30) = 3.54, $p.$ < .03]. Shown are means and standard errors of the mean. Star represents $p.$< .05.

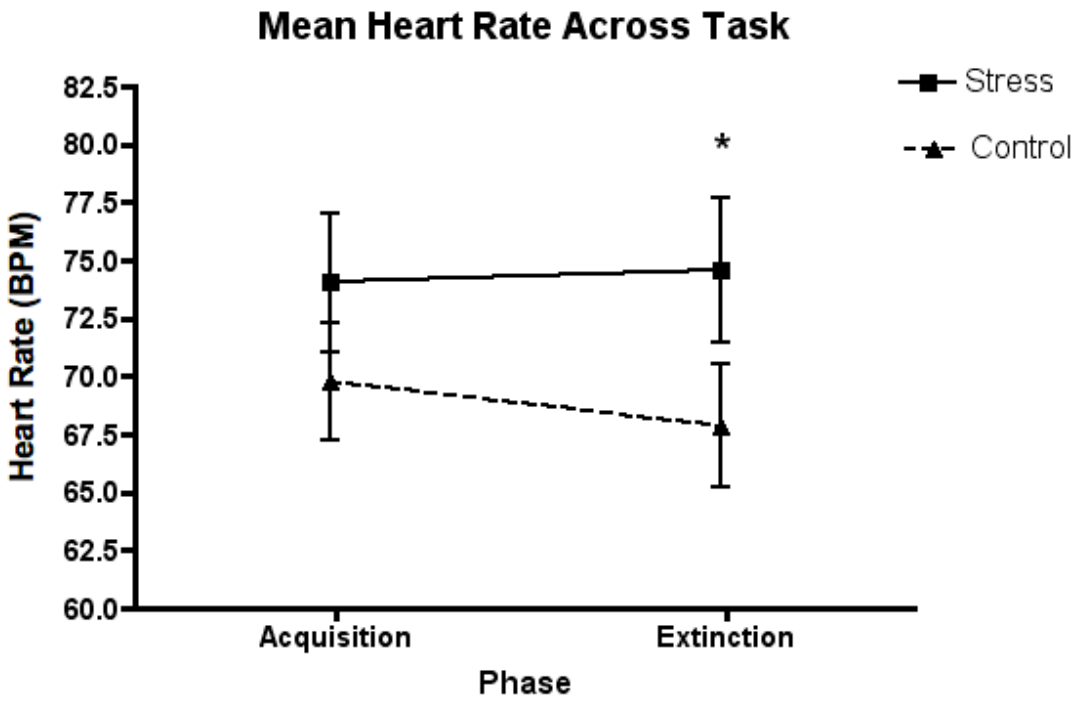


Figure 13. Mean heart rate at Acquisition and Extinction phase for Stress vs. Control group.

Independent of condition, subjects of the Stress condition (squares-full line) showed increased heart rate at Extinction relative to subjects of the Control condition (triangles-dashed line). Star indicates *p.*< .05.

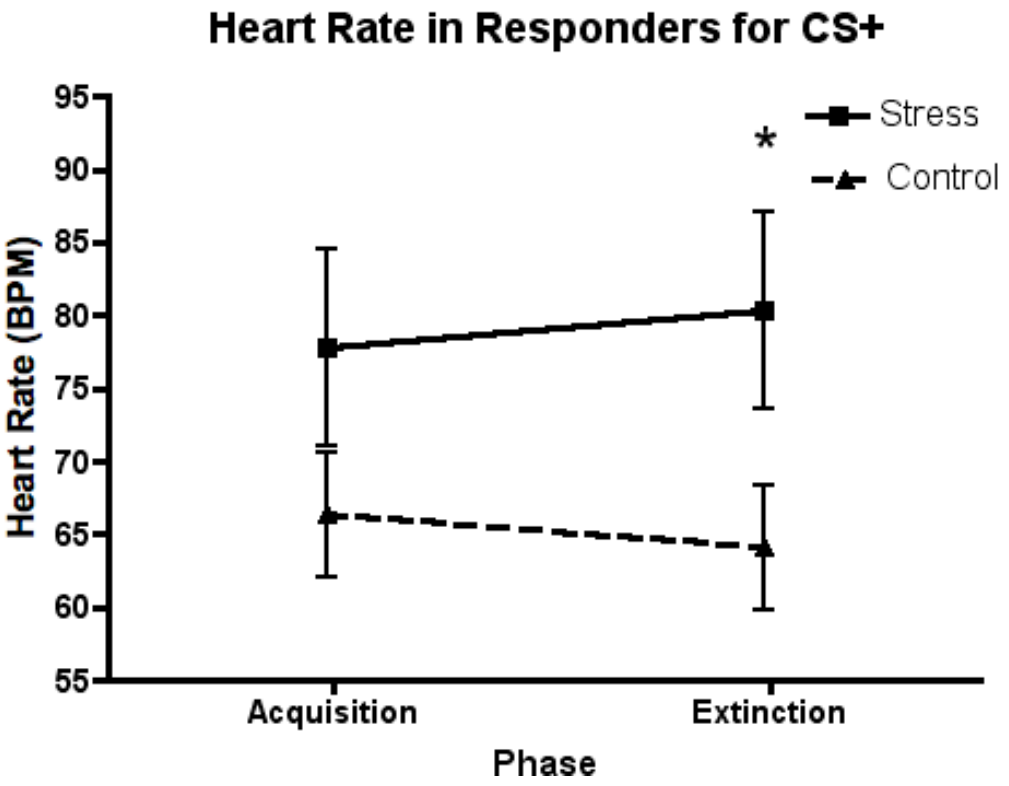


Figure 14. Mean heart rate in Responders for CS+ only.

Analyses reveal a difference in heart rate between subjects of the Stress condition (squares-full line) and subjects of the Control condition (triangles-dashed line). Error bars represent standard error of the mean. Star represents *p.*< .05.

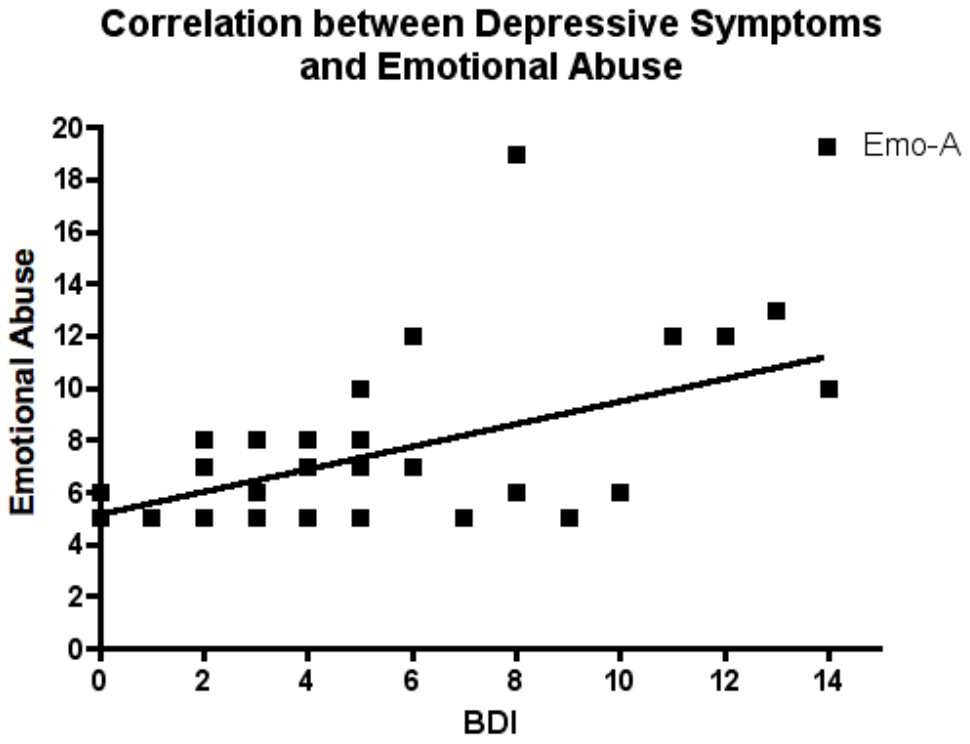


Figure 15. Correlation Depression and Emotional Abuse

Correlation across all subjects between depressive symptoms assessed with the Beck Depression Inventory and Emotional Abuse assessed with the Childhood Trauma Questionnaire, $r^2 = 0.37$.

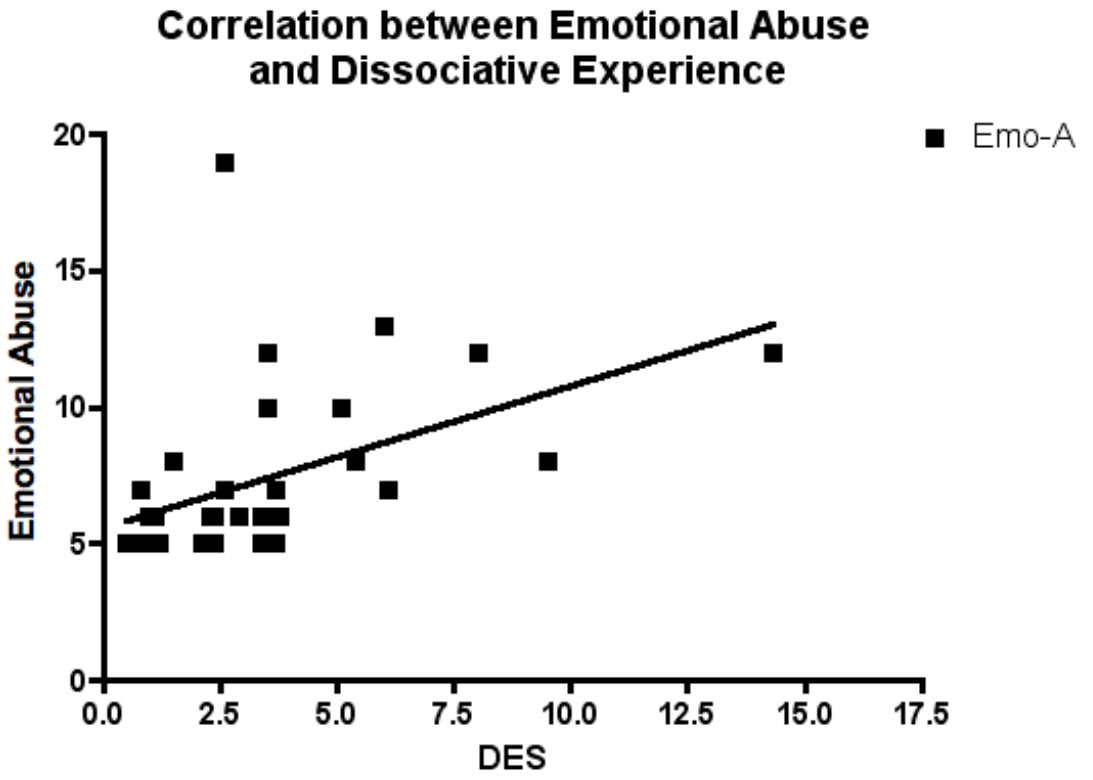
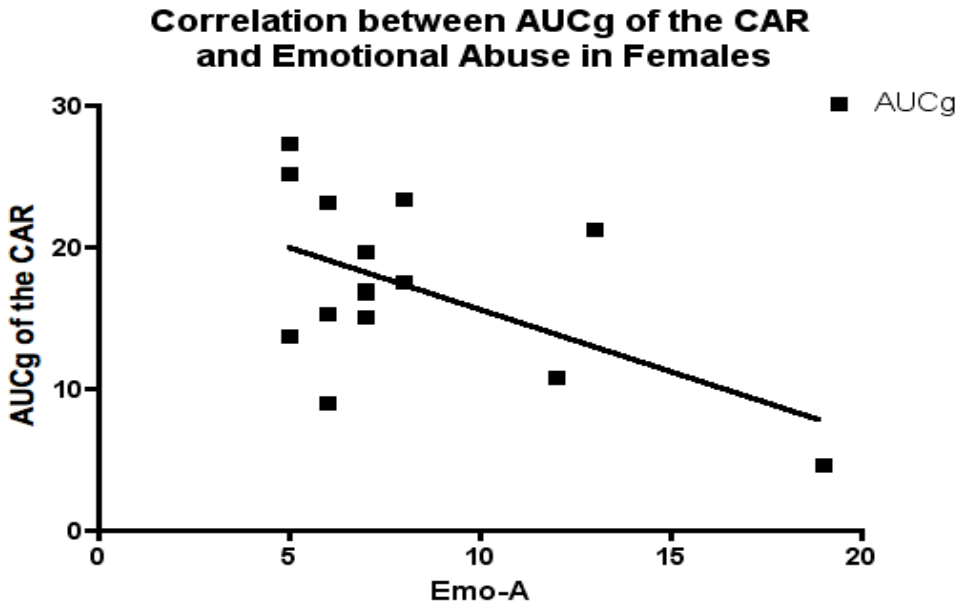


Figure 16. Correlation Dossociative experience and Emotional Abuse Correlation between Dissociative experience assessed with the Dissociative Experience Scale and Emotional Abuse assessed with the Childhood Questionnaire, $r^2 = 0.44$.

(A)



(B)

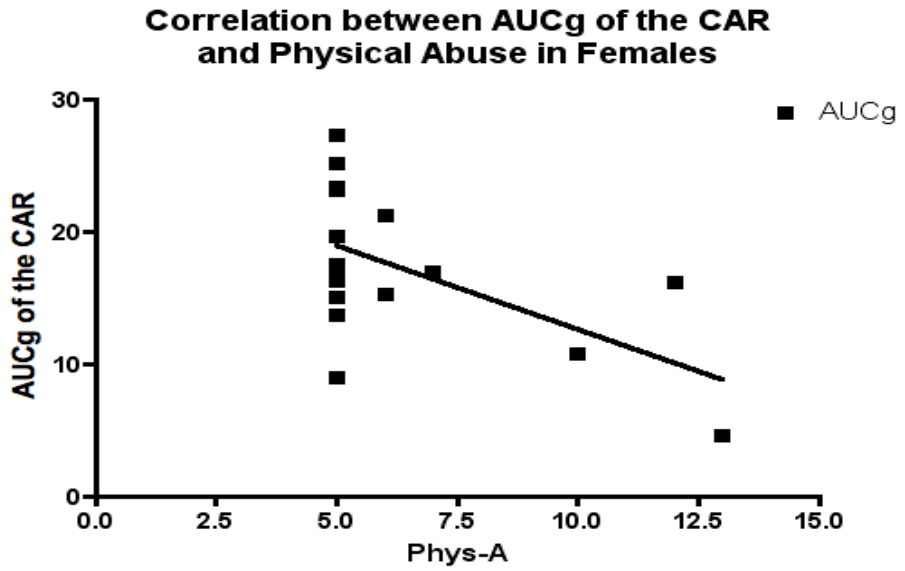


Figure 17. Correlation CAR and Childhood Abuse

Correlations between AUCg of the CAR and (A) Emotional and (B) Physical Abuse measured with the Childhood Trauma Questionnaire in Female subjects only.

	Stress N= 20	Control N = 20	*p.*
Age	21.68 (.80)	23.76 (.73)	.07
BDI	5.79 (.86)	4.20 (.87)	.20
STAI-S	50.94 (.66)	51.16 (.58)	.81
STAI-T	49.06 (.70)	47.85 (.47)	.15
DES	4.65 (.99)	2.52 (.41)	.06
CTQ-Emoa	7.11 (.52)	7.25 (.82)	.89
CTQ-Physa	6.11 (.45)	5.67 (.39)	.46
CTQ-Sexa	6.16 (.85)	5.81 (.53)	.73

Table 2. Socio-demographic data

Means and standard error of the mean; BDI = Beck Depression Inventory; STAI = Spielberger State and Trait Anxiety Inventory (S = State, T = Trait); DES = Dissociative Experience Scale; CTQ = Childhood Trauma Questionnaire; Emoa = Emotional Abuse; Physa = Physical Abuse; Sexa = Sexual Abuse.

Chapter 4: *Cortisol Awakening Response in civilians exposed to trauma; a community-based study*

1. INTRODUCTION

Post-traumatic stress disorder (PTSD) is a DSM-IV-TR (APA, 2004) anxiety disorder that is characterized by recurring intense fearful reactions, avoidance behavior and general hyperarousal, following exposure to a traumatic event. The current model for studying PTSD is based on pavlovian fear-conditioning (R. K. Pitman, 1989) (R.K. Pitman, et al., 2001). Studies of fear conditioning in humans emphasize the importance of the hypothalamic-pituitary-adrenal (HPA) axis in learning and expression of fear (Grillon, et al., 2006; Jackson, et al., 2006; Merz, et al., 2010). The HPA-axis gets activated in response to stress and an increase in its activity results in the release of cortisol from the adrenal cortex. The main effect of cortisol is to enable additional release of energy, thus facilitating the "flight or fight" response to threat. Furthermore, it is known to modulate declarative emotional memories (Buchanan & Lovallo, 2001; Cahill, et al., 2003; Cahill & McGaugh, 1996; Maheu, et al., 2004), a function that is altered in PTSD.

Because of its multi-systemic action, both centrally and peripherally, cortisol and the HPA-axis have been investigated as a potential biomarker for PTSD (Resnick, et al., 1997; Yehuda, 2002; Yehuda, et al., 2009). However, conclusions on the effect of cortisol on the etiology and maintenance of the disorder are limited by a few factors. One factor is the exact methodology used to collect and analyze cortisol. Some studies have used plasma cortisol (J. A. Golier, et al., 2006, 2007; Resnick, et al., 1997; Resnick, et al., 1995; Song,

et al., 2008), which allows for the comparison of free (active) versus bound (inactive) cortisol, a technique limited by the need for laboratory context and medical staff for collection of the samples. Another technique used is the collection of urinary cortisol, which allows for the measurement of total amounts of cortisol produced over a period of time (Bierer, et al., 2006; Glover & Poland, 2002; Murphy, 2003). This, however, limits the capacity to show the dynamic course of the hormone in response to specific stimulation. Lastly, some studies have used measurements of free salivary cortisol, a non-invasive sampling technique that can be used repeatedly to illustrate the dynamic changes in the hormone in response to environmental changes. In sum, it is hard to draw exact comparisons between studies since their cortisol sampling methods differs significantly.

Another limitation deals with the heterogeneity of the populations studied. Most studies have investigated status of cortisol in individuals suffering from chronic PTSD, in a retrospective design. The obvious limitation to these studies is that no clear causative links can be drawn between cortisol and the clinical status. Many confounding factors can further affect HPA-axis regulation from the moment of trauma until the beginning of systematic study, which sometimes occurs decades later.

Despite these limitations, cortisol continues to be one of the most promising biomarkers in PTSD research, and the Cortisol Awakening Response (CAR), as a naturally occurring stimulation response, received increased attention. As mentioned in the previous

chapters, the CAR has been a validated tool used to evaluate the integrity of the HPA-axis integrity (Clow, et al., 2004; Fries, et al., 2009; Kudielka & Wust, 2010). Previous studies have indicated that an abnormal CAR may be related to developmental risk factors for psychopathology (Dedovic, et al., 2010; Engert, Efanov, Dedovic, Dagher, et al., 2010). An early study using saliva samples looked at differences in pediatric PTSD and found no significant differences at awakening (Carrion, et al., 2002). This finding has been replicated in another sample of children exposed to trauma (Suglia, Staudenmayer, Cohen, & Wright, 2010). One study has shown that the CAR was actually increased in nurses suffering from chronic PTSD (Metzger, et al., 2008). However, other studies have found no link between the CAR and PTSD diagnosis (Johnson, Delahanty, & Pinna, 2008; van Zuiden, et al., 2010). Adding to the debate, in a recent study, free salivary cortisol samples were used to examine the CAR in individuals suffering from chronic PTSD, compared to groups of trauma-exposed individuals who did not suffer from PTSD, and to healthy individuals. This study revealed that a blunted CAR was associated with chronic PTSD diagnosis, but not exposure to trauma alone (Wessa, et al., 2006). However, no study so far has examined the integrity of the HPA-axis in civilians exposed to a first traumatic event, during adulthood, in a longitudinal design, in order to examine potential changes in HPA-axis activity with the course of illness.

In the current study, we proposed to examine the CAR of trauma-exposed subjects at one month after trauma exposure and once more a year after trauma exposure. Based on the study from

Wessa et al. and studies showing the stability in time of the CAR, we hypothesized that the CAR would be stable over time, but that individuals suffering from PTSD at both time points would present with a lower CAR compared to individuals who never developed PTSD, and individuals who remitted from PTSD between the two assessments.

2. METHODS

Thirty individuals exposed to a traumatic event were recruited from the emergency rooms of various hospitals in the Montreal region. All subjects were assessed for symptoms between four and six weeks of trauma exposure, and then at follow-up twelve months post-trauma. From this follow-up, three groups were formed: Chronic PTSD (PTSD+ at both time points, n = 12), Trauma-Exposed Without PTSD (PTSD- at both time points, n = 11), and Remission (PTSD+ at one month post-trauma, PTSD- at 12 months assessment, n = 7).

All participants met the DSM-IV A1 and A2 criteria for trauma exposure (APA, 2004). Events included motor vehicle accidents, as well as physical assault, sexual assault, and industrial accidents. Subjects were excluded if they had a history of head injury or loss of consciousness as a result of the traumatic event, neurological disorders (e.g. epilepsy) or a current/history of Axis 1 disorder (e.g. bipolar disorder, schizophrenia, major depression, alcohol or substance abuse or dependence). Another group of 14 healthy age-matched individuals without history of trauma exposure or Axis 1 disorder was recruited through newspaper ads. This group was asked to provide

samples at two time points mirroring the two assessment times of the clinical groups (11 months apart).

The self-report Impact of Event Scale-Revised (Brunet, St-Hilaire, Jehel, & King, 2003) was used to provide a dimensional assessment of PTSD symptoms experienced in the previous week. The Clinician-Administered PTSD Scale (Blake, et al., 1995), an interviewer-based structured interview, was used to determine PTSD diagnostic status. In addition to meeting the DSM-IV criteria for PTSD (APA, 2004), a minimum score of 42 on the CAPS was required to ensure that all subjects' PTSD diagnosis was unequivocal.

Cortisol was sampled using cotton-based salivettes (Sarstedt Inc., Quebec City, Canada), at six points during the day both at one month and twelve months post-trauma: Awakening, Awakening +30, Awakening +45, Awakening +60, Awakening + 8 hours, and Awakening +16 hours. All samples were frozen (-74 °C) upon reception and sent for analyses. All samples were analyzed through fluorescenceimmuno assay.

Initial analyses showed effects only for the first hour after awakening, thus only the first four samples were kept. A mixed-design analysis of variance (ANOVA) was performed with Time-point and Samples (awakening, awakening +30 mins, etc) as within-subject factor and Group as a between-subject factor, with Sex included as a cofactor. Also, Area Under the Curve (AUC) was computed according to the formula found in (J. C. Pruessner, et al., 2003), in order to better examine the dynamics of the CAR. A mixed-design analysis of

variance was performed using the AUC_g and AUC_i as outcome measures, with time of sample (one vs 12 months) as the within factor and group as the between subjects factor. Additionally, exploratory analyses for correlations between clinical scores and cortisol levels (AUC_g & AUC_i) were performed, using Bonferroni's corrections for multiple comparisons.

3. RESULTS

Statistical analyses revealed that groups did not differ in terms of age [F (2, 27) = .99, p. < .38]. Significant differences between groups were observed for PTSD severity [F (2, 34) = 30.99, p. <.00], peritraumatic distress [F (2, 34) = 3.18, p.< .06] and depression symptoms [F (2, 34) = 6.17, p. <.004], but not for peri-traumatic dissociation [F (2, 34) = .336, p. < .72]. Table 3 summarizes socio-demographic variables of the clinical groups.

Analyses of variance revealed no significant interaction between Time-Point, Samples and Group. Also, our analyses revealed no main effect of Time-Point. Lastly, a trend was observed for a main effect of Group [F (3, 39)= 2.66, p. < .06]. When we examined the clinical groups only, our analyses revealed a significant Sample by Group interaction [F (2, 26)= 2.50, p. < .05; Figure 18].

Analyses of the AUC revealed no significant interaction between time of sampling and group on the AUC_i [F (2, 27) = 1.65, p.< 0.21]. However, there was a significant main effect of group [F (2, 27) =

3.39, p. <.05], congruent with our Sample by Group interaction within the clinical groups. Tukey's post-hoc analyses revealed that the trauma-exposed group without PTSD showed a bigger increase in CAR at both time points, compared to the two other groups (Figure 19). Concerning the AUC_g, we did not observe any interaction between time of sampling and group [F (2, 27) = .21, p.<.82], but we did observe a trend for a main effect of group [F (2, 27) = 2.77, p. <.08]. Post-hoc analyses computed with the software G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) revealed that, considering our sample size and a power of 1-ß of 0.90, the effect size of the AUC_i difference detected was of f = 0.35, which is considered to be between medium and high.

4. DISCUSSION

In the current study, we examined the cortisol awakening response in individuals recently exposed to trauma at two time points, within one month of trauma and at 12 months follow-up, compared to the CAR of healthy individuals. When investigating specifically the clinical groups, a significant sample by group interaction emerged. Examination of the Area under the Curve revealed a significant group effect in the AUCi where the group of subjects exposed to trauma that never developed PTSD showed greater free salivary cortisol in response to awakening than both the chronic PTSD and remission group. These differences appeared specifically for the second assessment.

Our results show an interesting pattern, in that they point to a possible role of cortisol and, more broadly, of the HPA-axis in the capacity to resist the deleterious effects of trauma exposure. The current results show that, among the subjects exposed to trauma, only those subjects who never developed significant PTSD symptoms had a normal CAR, whereas developing early PTSD, independent of future remission or not, was associated with a lower CAR. This may suggest that the HPA-axis and cortisol may help modulate early resilience rather than be involved at later stages of the disorder. We might therefore conceptualize resilience as the early capacity to mount a consistent and persistent biological stress response, and thus acquire and consolidate extinction learning. Studies have shown how extinction learning is dependent on the hippocampus in the early stages (Knight, et al., 2004), whereas consolidation and retention of this learning is mostly dependent on the ventromedial prefrontal cortex (M.R. Milad, et al., 2007). Both regions show a high concentration of glucocorticoid receptors and are involved in the top-down regulation of the HPA-axis (Buchanan, et al., 2004; Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). It is thus possible that both CNS structures and hormones might interact, beginning at the time of trauma exposure, to suppress the symptomatic fearful reactions typical of PTSD. A normal and stable CAR would therefore contribute to resilience.

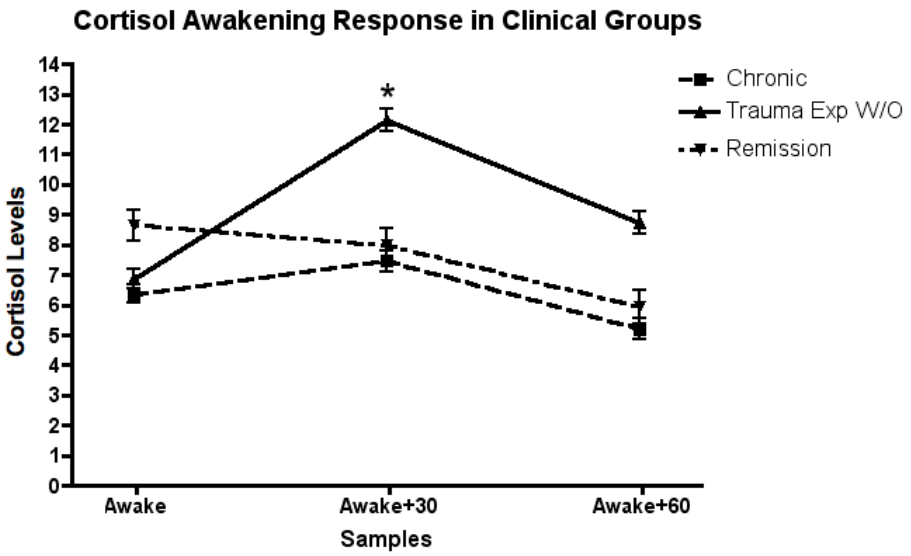
The HPA-axis is a well-known modulator of learning, as has been observed in many different tasks in humans (Maheu, et al., 2004; Maheu, et al., 2005; Stegeren, et al., 2007; Zorawski, et al., 2006;

Stark, et al., 2006). Since the current model for studying PTSD is based on the learning theory of fear conditioning and fear extinction, our results indicate the importance of considering cortisol as a potential modulator of resilience, especially considering the results from chapter 3 of the current thesis, which illustrated that an increase in cortisol may help enhance extinction learning. The current literature agrees that the awakening response, while not a perfect method, is still a reliable index of the integrity of the HPA-axis and of its capacity to respond to challenges and threats (Fries, et al., 2009; Kudielka & Wust). However, it must be recalled that the HPA-axis, like most biological system, can adjust and up- or down-regulate its activity, depending on contextual factors. A recent study from our laboratory has shown that the awakening response can be influence as early as childhood through the bonding with parents (Engert, Efanov, Dedovic, Dagher, et al.). It may be that early experiences could influence the HPA-axis to confer either vulnerability or resilience to stressors and traumatic experiences (Yehuda, et al., 2010), a model that has been extensively investigated in rodents (Bagot, et al., 2009; Zhang, et al., 2006). This developmental model is supported by other studies that show biological risk factors to developing PTSD (Gilbertson, et al., 2002). Considering this property, our study underscores the need for more longitudinal investigations with repeated measures, allowing for a better understanding of the dynamics of this system following exposure to trauma, as well as the need to investigate developmental factors in individuals exposed to trauma. This approach may help to better characterize the impact of early experiences on the reaction to trauma in adulthood.

Our study did present some limitations. First, we did have a small number of subjects, especially in the remission group. Second, both genders were not equally represented in each group, which reflects what has been observed in epidemiological studies, but which prevents a full comparison. Also, we did not screen our female subjects for use of oral contraceptives, which may influence the regulation of cortisol. Last, we did not inquire about the quality of sleep in our participants, which may have modulated the cortisol awakening response. However, despite those limitations, we do feel that our study shows the importance of considering cortisol as a resilience factor to trauma.

In sum, our data underlies the importance of more research that specifically examines the evolution of symptoms in individuals recently exposed to trauma. If resilience and remission are distinct biological processes, then a special attention should be dedicated to teasing these concepts apart at the biological levels. Our results seem to indicate that they are, but more data is needed to fully understand these processes. Also, considering the close interaction between cortisol and the central nervous system, studies should focus on measuring the modulatory role of basal cortisol as well as reactive cortisol on the consolidation and expression of symptoms. This way, we may better understand how to adjust therapies at various stages of the disorder, in order to compensate or help hormones lessen the burden of symptoms.

(A)



(B)

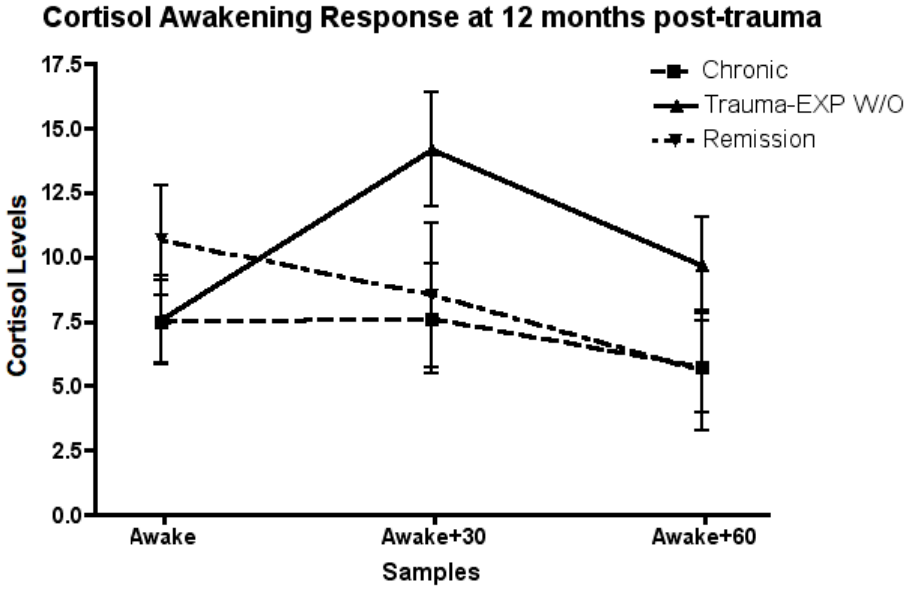


Figure 18. Cortisol Awakening Response for clinical groups

(A) One month and (B) twelve months post-trauma. Analyses indicate a significant difference at the awakening +30 minutes sample: the Trauma-exposed without PTSD (Trauma-EXP W/O) group show greater increase compared to subjects suffering from Chronic PTSD (Chronic) and subjects in remission (Remission).

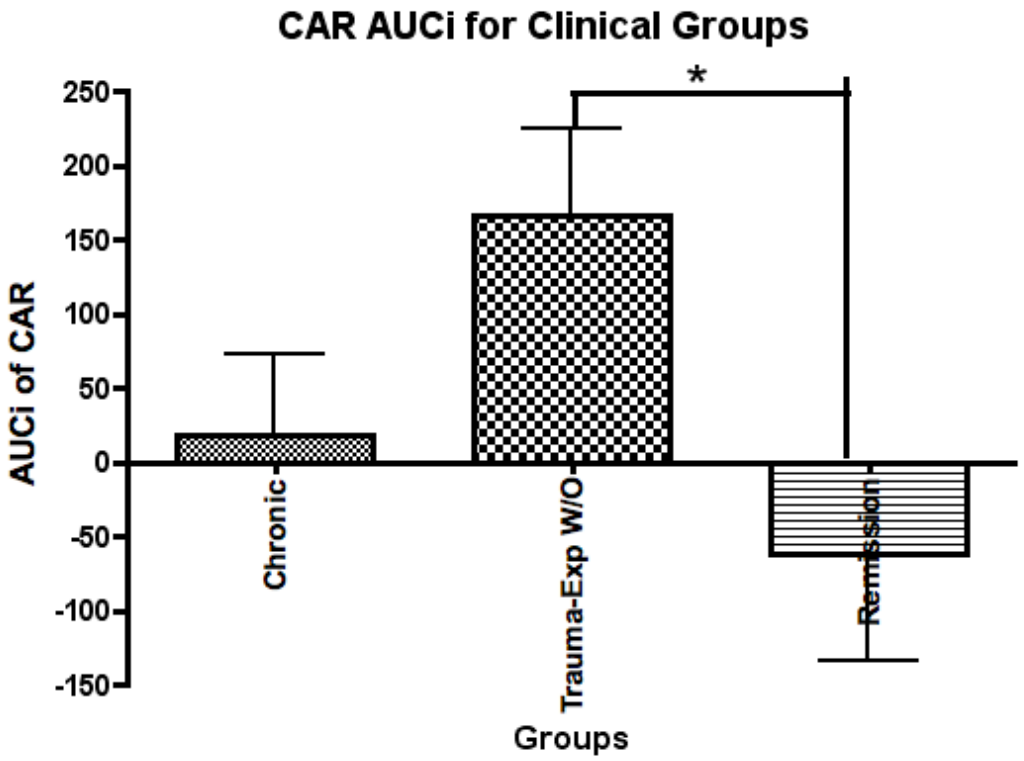


Figure 19. Area Under the Curve increase of the CAR for clinical groups

Results show a significant difference in increase between the Trauma-Exposed without PTSD subjects and those in Remission, independent of the sample time (one month and twelve months post-trauma).

		CHRONIC	TRAUMA-EX	REMISSION	p.
AGE		29.25 (9.72)	25.45 (4.89)	29.00 (8.08)	.471
CAPS	T1	66.83 (16.13)	25.27 (10.04)	59.71 (19.28)	.000**
	T2	53.33 (20.42)	12.55 (9.59)	13.71 (8.46)	.000**
IES-R	T1	52.58 (22.61)	24.27 (13.37)	34.71 (21.84)	.006**
	T2	50.45 (26.87)	22.45 (12.38)	35.50 (26.13)	.022*
PDI	T1	27.58 (9.92)	19.45 (6.64)	22.57 (5.59)	.064
	T2	23.00 (13.31)	16.00 (6.99)	23.83 (9.60)	.213
PDEQ	T1	28.67 (10.14)	22.64 (8.39)	20.71 (7.52)	.136
	T2	26.36 (9.45)	23.00 (11.06)	22.33 (11.07)	.672
BDI	T1	11.42 (6.02)	2.18 (2.09)	5.00 (4.62)	.000**
	T2	7.36 (6.53)	2.27 (2.97)	1.57 (2.15)	.017*

Table 3. Socio-demographic data for clinical groups

CHRONIC=Chronic PTSD, TRAUMA-EX=Trauma-exposed without PTSD, REMISSION= PTSD at time point 1, no PTSD at time point 2; CAPS = Clinician-Administered PTSD Scale; IES-R = Impact of Event Scale

revised; PDI = Peritraumatic Distress Inventory; PDEQ = Peritraumatic Dissociative Experience Questionnaire; BDI = Beck Depression Inventory; T1 = one month post-trauma, T2 = twelve months post-trauma; all data shown are means and standard error of the mean.

Chapter 5: *Cortical Thickness in Individuals Exposed to Trauma; a longitudinal study of a civilian sample*

1. INTRODUCTION

Post-traumatic Stress Disorder (PTSD) is a DSM-IV (APA, 2004) anxiety disorder that is characterized by flashbacks, intrusive memories and hyperarousal symptoms that persist following exposure to a traumatic experience. These symptoms, while present in a certain percentage of individuals exposed to trauma, tend to abate in time, either on their own or through therapy (Breslau, 2001). In a sub-sample of individuals, however, the symptoms resist attempts at treatment and may persist for years, provoking intense suffering and, oftentimes, secondary co-morbid conditions such as Major Depression and Alcohol Abuse (Brunello, et al., 2001).

In order to better understand the cause and development of the abnormal fearful reactions, most researchers use a model based on fear conditioning (R. K. Pitman, 1989). According to this model, PTSD is considered to be an exaggerated fear conditioning acquisition that is overly consolidated through the action of stress hormones and catecholamines. Following this initial stage, individuals who suffer from PTSD are thought to be unable to successfully learn and recall extinction of the initial fearful, traumatic event. This model has been very useful to determine neuroanatomical regions of interest that may be responsible for the emergence and persistence of symptoms. Among these regions, a specific triad has been extensively identified, based on animal studies (J. E. LeDoux, 2000). While a complete review of the studies conducted so far is beyond the scope of this study, the emerging model suggests that an overactive amygdala would be

responsible for the acquisition and maintenance of symptoms, while a hypoactive and smaller bilateral anterior cingulate cortex (ACC) and hippocampus would underlie the inefficient extinction learning and thus incapacity to suppress symtoms (Armony, et al., 2005; J. D. Bremner, Vythilingam, Vermetten, et al., 2003; Shin, Orr, et al., 2004; Shin, Shin, et al., 2004; Shin, et al., 2005; Smith, 2005; Woodward, Kaloupek, Streeter, Martinez, et al., 2006). Interestingly, as mentioned in Chapter 1 of this thesis, these structures are also involved in the regulation of the HPA-axis and stress reactivity. As shown in Chapter 4, the HPA-axis seems to be involved in PTSD: cortisol levels may be associated with resilience.

However, most of these studies share common limitations with respect to design and sampling. More specifically, studies examining individuals suffering from PTSD have most often used a retrospective design, scanning participants many years after the onset of the disorder (Smith, 2005; Hull, 2002). This prevents any inference as to the causality link between brain structures and symptoms; any result found could be interpreted as either a cause or a consequence of the disorder, which is a common line of argument in the current literature. Another common limitation to the previously mentioned studies is the cross-sectional design: most studies report results based on a single data acquisition session. This prevents any conclusions to be drawn as to the effect of time on the interaction between brain and symptoms. Lastly, previous studies have focused on specific homogeneous samples, such as veterans or adult survivors of childhood abuse. Results obtained from such samples are limited with respect to

generalization to the civilian population at large. Trauma exposure in the civilian population is most often related to a single discrete event, such as motor-vehicle accident or assault, which stands in contrast with the chronic stressful exposure of a presence in a war zone or childhood abuse. Furthermore, childhood abuse may influence the normal developmental trajectory. Few of the previously mentioned studies have studied an adult sample exposed to a first discrete traumatic event, in a quasi-prospective longitudinal design.

Despite these limitations, the study of the fear-circuit mentioned above has allowed confirming some mechanisms involved in the emergence and persistence of the fear-related symptoms of PTSD. They also mirror effectively the findings observed in rodents in a fear-conditioning paradigm (J. E. LeDoux, 2000). Furthermore, they provide interesting avenues for treatment of these fear-related symptoms (Brunet, et al., 2007). However, PTSD is a complex psychiatric condition that includes other processes not directly related to the fear circuitry (Hathaway, Boals, & Banks). These additional dimensions of PTSD, such as shame and guilt, suggest that other brain regions may be involved in the development and maintenance of the disorder. Additionally, the cognitive element of PTSD indicates that some regions outside the limbic system, mainly in the dorso-frontal area, may also be involved. These elements are also near impossible to model in rodents, preventing MRI studies from investigating precise regions that may be affected. In human subjects, previous methods of assessing the structural integrity of the central nervous system limited the possibility of examining structures that do not belong to a

specific region of interest like the fear circuit. Specifically, automated methods of global structural assessment such as Voxel-Based Morphometry were reported to yield significant differences that were not related to the actual structural integrity (Corbo, Clément, Armony, Pruessner, & Brunet, 2005). The alternative was to segment brains using semi-automated or fully manual tracing. The down side of this approach is the immense time invested necessary to segment every brain.

Recently, advances in neuroimaging protocols have allowed the validation of a new fully automated technique, Cortical Thickness, which is meant to examine the integrity of the cortex along the whole cerebrum (Lerch & Evans, 2005) and has already been used to study normal aging (Chen, He, Rosa-Neto, Gong, & Evans, 2011). The purpose of the current study was to correlate the cortical thickness of individuals exposed to trauma with severity of symptoms at both one month and twelve months post-trauma. Based on previous literature, we hypothesized that symptom severity would be negatively correlated with cortical thickness of the Anterior Cingulate Cortex. We also ran exploratory analyses of the whole cerebrum in relation with symptom severity.

2. METHODS

2.1 Participants

Thirty-eight trauma-exposed individuals were recruited from various emergency rooms of hospitals of the Greater Montreal region,

Canada. Potential subjects were excluded if they had a prior history of Axis-I Disorder, head injury resulting in a loss of consciousness, or a history of neurological disorder. All subjects met the DSM-IV-TR (APA, 2004) criteria A1 and A2 for exposure. Once they contacted our team, subjects were scheduled for a first clinical assessment and MRI exam, within 6 weeks of the traumatic event. Eleven months after the initial assessment, subjects were contacted for a follow-up clinical and MRI assessment. Participants gave written informed consent and received moderate financial compensation for their time and effort. The study was approved by the Douglas Institute Research Ethics board.

2.2 Clinical Assessment

To assess the severity of symptoms, the Clinician-Administered PTSD Scale [CAPS (Blake, et al., 1995)] and Impact of Event Scale [IES-R (Brunet, et al., 2003)] were used. In order to control for potential co-morbid depressive symptoms, the Beck Depression Inventory [BDI (Beck, et al., 1961)] was used for all subjects. These questionnaires were administered at both time points.

2.3 MRI Image Acquisition

All scans were performed at the Montreal Neurological Institute (MNI) using a 1.5T scanner (Siemens Vision, Erlangen, Germany). T1-weighted image scans using a three-dimensional (3-D) spoiled gradient echo acquisition with sagittal volume excitation (echo time [TE] = 10, repetition time [TR] = 18, flip angle = 30°, 140 contiguous 1-mm

sagittal slices) and a rectangular field of view (FOV) of 256 mm (superior-inferior [SI]) by 256 mm (anterior-posterior [AP]) were acquired to guarantee a high resolution for structural analysis.

2.4 Cortical Thickness Analysis

All T1 raw images were first corrected for non-uniformity of signal (Sled, Zijdenbos, & Evans, 1998), normalized into standard stereotaxic space (Collins, Neelin, Peters, & Evans, 1994) and classified into grey, white matter and cerebrospinal fluid (Zijdenbos, Forghani, & Evans, 2002). These first steps allow for controlling for differences in head size across subjects. Cortical Thickness was computed following the protocol validated by (Lerch & Evans, 2005) (Lerch, et al., 2008). These steps were done using the Graphic User Interface AutoCort developed in our laboratory by a computer programmer (T.B.). This software allows the automated computation of all steps and extractions described below on external servers. Briefly, cortical thickness allows the creation of inner and outer cortical surfaces using a surface extraction algorithm (ASP). This algorithm identifies the border between white and grey matter, as well as grey matter and CSF. This computation is done along over 81 000 polygons. The resulting images are then extracted using the constrained Laplacian based automated segmentation with proximities (CLASP) algorithm (Kim, et al., 2005) and blurred using a 20-mm surface-based diffusion kernel. This program therefore allows for an automated reliable assessment of the thickness of the whole cerebrum. However, sub-cortical structures were not assessed.

The thickness computed for each subject was then regressed against symptom severity as assessed by the CAPS and IES-R with the use of AutoCort, with Age and Depression scores included as a covariate. All analyses were controlled for multiple comparisons using a false discovery rate (FDR) correction.

All thickness data were viewed using the software Brain-view, which allows examination of cortical thickness analyses in tri-dimensional space and identification of loci of significant results. Correlations between clinical data were done using the software SPSS 16.0 for MacIntosh computers.

3. RESULTS

3.1 Socio-demographic variables

All socio-demographic data can be found in Table 4. Briefly, Age did not correlate with symptoms severity as assessed by either CAPS or IES-R (all $p.> .30$). An independent t-test revealed a significant effect of Sex on symptom severity assessed with the IES-R at both time points [$t(37) = 1.99$, $p.< .05$; $t(37) = 2.37$, $p.< .02$]; females reported significantly more severe symptoms compared to males (see Figure 1). However, there was no significant difference between sexes for symptoms severity as assessed with the CAPS (all $p. > .20$). Finally, depression scores assessed by the BDI were also highly correlated with PTSD symptom severity assessed both with CAPS and IES-R at both time points (all $R > .40$, all $p. <.01$; see Table 5).

3.2 Cortical Thickness Analyses

At one month post-trauma, a significant negative association between self-report symptoms and thickness of the ACC was found in both the right [t (37) = -3.42, $p.$ < .003, Brodmann Area 24; Figure 21 A] and left [t (37) = -3.29, $p.$ < .003, Brodmann Area 24; Figure 21 B] hemispheres. Further, self-report symptoms severity was negatively correlated with cortical thickness of the left ventral temporal pole [t (37) = -4.31, $p.$ <.000, Brodmann Area 38; Figure 22]. Last, a significant positive association was detected between symptoms severity assessed by the CAPS and cortical thickness of the right anterior frontal pole [t (37) = 5.45, $p.$ < .000, Brodmann Area 10; Figure 23].

However, when correlating thickness and symptoms severity at the 12-months post-trauma time point, no significant association was detected.

4. DISCUSSION

In the current study, we examined the link between cortical thickness across the whole cerebrum and PTSD symptom severity in a community-based sample of individuals exposed to trauma. We examined this relationship at two time points, i.e. one and twelve months post-trauma. Congruently with our hypothesis, we found a significant negative correlation between thickness of the ACC and self-

reported symptom severity at first assessment. Interestingly, this relationship was not present at the second assessment. Also, we did not find this relationship with symptom severity assessed by a trained clinician at either time points. Further, our exploratory analyses revealed two novel regions that shared strong associations with symptoms severity. First, there was a significant negative correlation between self-reported symptoms and ventral temporal area. The second association was found to be a positive correlation between severity of symptoms assessed by a clinician and thickness of the right anterior frontal area. These associations are new findings that have never been reported. Again, these associations were present only for the first assessment and did not translate to analyses performed for twelve months post-trauma.

Concerning the ACC, there is extensive literature linking this region to symptoms of PTSD. Functional MRI studies have shown a decrease in activity of the ACC that correlated with an increase of activity of the amygdala (Shin, Orr, et al., 2004; Shin, et al., 2005; J. D. Bremner, et al., 2004). The common interpretation of these findings is that the ACC may be responsible of suppressing the excitatory fearful responses triggered by the activity of the amygdala. This action would be exerted through the excitation by the ACC of intercalated inhibitory GABAergic neurons in the amygdala (Rodrigues, et al., 2009). In terms of the fear-conditioning model, a hypoactive ACC would be unable to support the extinction learning that is hypothesized to inhibit the fearful response post-conditioning, i.e. post-trauma (Shin, et al., 2005). This view was supported by an

interesting study that examined the link between cortical thickness and fear extinction in healthy individuals (M.R. Milad, et al., 2007). Specifically, the authors indicated that the ventro-medial prefrontal cortex was associated with the recall of extinction, not the acquisition of it. Since the current model of PTSD views this disorder mostly as a failure to retain and express extinction learning, an hypoactive and under-developed ACC could explain the inability of individuals suffering from this disorder to exert voluntary control over their fearful reactions. This model has been supported by structural data investigating the ACC in PTSD. One early study by Rauch and colleagues (Rauch, et al., 2003) found a smaller volume of the ACC in nurses suffering from PTSD compared to nurses exposed to trauma who did not develop PTSD. This finding has been replicated by Kitayama and colleagues (Kitayama, et al., 2006) in abuse-related PTSD, as well as by Woodward and colleagues (Woodward, Kaloupek, Streeter, Martinez, et al., 2006) in veterans exposed to combat. Our data supports this model by showing that a thinner and possibly under-developed ACC is associated with symptoms severity at the first assessment. However, it is interesting to note that this result was true only for the self-report assessment and not the clinician-administered one. Also, it must be noted that, while we found this association at the first time point, we did not replicate it at the later time point. The reason for this may be due to the decrease in symptom severity in a portion of our sample, which may have prevented the association from being detected. However, the mean severity of symptoms did not change significantly across our whole sample. A future study using a larger sample could investigate the association in a group design including

individuals who remit from the disorder. Also it may be possible that while the ACC is an important structure for the early acquisition of the disorder, its structure may not be associated with later persistence of symptoms. As mentioned above, recent evidence from epidemiological studies as well as factor studies on the model of PTSD are indicating that the memory-based, fear-conditioning model may account for earlier stages of the disorder, but may not be the best predictor of the persistence of the disorder in time (Shea, et al., 2010). This observation warrants further investigation of the ACC's integrity, both functionally and structurally, specifically in a longitudinal design in order to better account for a potential shift in time of the importance of this structure in the symptomatology of PTSD.

Our finding of a negative correlation between cortical thickness and the ventral temporal pole is an original discovery. A recent review (Olson, Plotzker, & Ezzyat, 2007) highlights the importance of the temporal pole in emotional processing. Specifically, the temporal pole is densely connected to the amygdala and the hypothalamus, two regions involved in PTSD, the former being hyperactivated (Armony, et al., 2005; Rauch, et al., 2000; Shin, Orr, et al., 2004) and the latter being at the origin of the stress axis, the hypothalamo-pituitary-adrenal axis, that is responsible for the secretion of cortisol (Chrousos, 2009; Korte, 2001; Kudielka & Wust, 2010). It has been previously observed that cortisol secretion in individuals with PTSD may be altered, although authors disagree as to the exact nature of the alteration (Chida & Steptoe, 2009; Fries, et al., 2009; Yehuda, 2002, 2009). In Chapter 4, we reported altered levels of cortisol in response to

awakening in individuals with Chronic PTSD as well as in subjects in remission, highlighting the importance of the HPA-axis and its central regulators in PTSD. Another important connectivity of the temporal pole is with the insula, a region that has been reported to be involved in fear conditioning (Tabbert, et al., 2010) and PTSD (Corbo, et al., 2005; Lanius, et al., 2004). Interestingly, lesion-based studies have found an important link between the temporal pole and social withdrawal, abnormal social behavior and general apathy (Olson, et al., 2007). These findings translate well into a model of PTSD where an abnormally developed temporal pole could be associated with the avoidance behavior / emotional numbing cluster of symptoms that seem to impact patients importantly (Shea, et al., 2010; Engdahl, et al., 2010). This finding argues for further investigation using functional brain imaging, to better evaluate the importance of the temporal pole function in the social and emotional aspects of PTSD. This line of investigation may have a great impact on help-seeking behavior and the effect of social support on symptom management.

Our finding of a positive correlation between symptom severity and anterior frontal cortex was unexpected. This region has never been reported to play a significant role in PTSD and seems more associated with purely cognitive processes. However, one possible explanation for its association with symptoms of PTSD may be its role in generating spontaneous mental activity, i.e. mental activity that is not a consequence of external stimuli, but related to internal processing (Ramnani & Owen, 2004). Considering the intrusive memories that are a hallmark of PTSD (R. K. Pitman, 1989; R.K.

Pitman, et al., 2001), this region may support the un-cued retrieval of those memories, especially in individuals that lack the capacity to inhibit them through the ACC. As is true with the temporal pole, our results underlie the importance of developing studies that may better investigate the role of structures like the anterior frontal area in PTSD, even if they do not belong to the fear circuit *per se*. It must be noted that cognitive function has been shown to be slightly altered in individuals with PTSD, especially attentional processes and immediate/short-term memory (Horner & Hamner, 2002). One recent study has shown that patients with PTSD may have a greater difficulty engaging in cognitive tasks (Daniels, et al., 2010). While this study did not identify the anterior frontal areas, the task used was a word memory task. Future studies should focus on tasks that target this region more specifically, in order to better explore its potential role in the emergence of symptoms as well as abnormal cognition.

Interestingly, our analyses did not reveal any significant associations at the second time point. This may be due to the fact that some subjects remitted in the 11 months that separated the two assessments. This would influence the general distribution of symptoms along the severity continuum, preventing us from detecting any linear association. However, as indicated above, the mean score of symptoms did not change across our sample: only a few subjects saw their scores decline. A larger sample would be necessary to investigate the link between remission and cortical thickness. Also, it may be that structural differences account mostly for the acquisition of the disorder and not for its maintenance in time. A last explanation is that

changes in the cortical organization occurred between the two assessment times, changing the pattern of associations between symptoms and cortical thickness. However, at this stage, we are unable to confirm the presence of such a change.

Our study did suffer from a few limitations. First, we did not control for the use of medication in our subjects to assess for potential effect of anti-depressants on changes of cortical thickness. Previous studies have highlighted the impact of such medication on some structures of the central nervous system (D. J. Bremner, et al., 2005). However, no reports have been published on actual changes in thickness related to medication, which doesn't mean that it may not have been a confounding factor, especially at the second time point. Also our sample showed a significant association between PTSD symptoms severity and depressive symptoms. This association occurs in general in patients suffering from PTSD (Brunello, et al., 2001). However, even if we controlled statistically for the effect of depression, it may still be limit our conclusions about the direct link between thickness and symptoms of PTSD. Lastly, our results are about the structural integrity of the cerebrum. We cannot draw any certain conclusion about the actual level of activity of these regions.

Our study was, to our knowledge, the first to investigate the early post-trauma association between cortical thickness and PTSD symptom severity in a sample of civilians exposed to a first traumatic event. We were also the first to offer a follow-up at 12 months post-trauma. Our results highlight once more the importance of the

cingulate region. But the strong associations found in the ventral temporal pole and anterior frontal area indicates that regions outside the typical fear-network may be strongly associated with PTSD symptoms. This would argue for new investigation of the function of these regions in relation to symptoms of PTSD that are not exclusively based on the processing of fearful stimuli, but that relate more to the avoidance/emotional-numbing cluster. Alterations of social behavior and cognitions in PTSD are already important targets of specific therapies designed for PTSD, e.g. Cognitive Processing (Solomon & Johnson, 2002). This line of therapy has been shown to be as effective at reducing symptoms as the various forms of exposure therapy, which are based on the process of extinction learning. Considering this, alterations of structure and function of brain regions such as the ones reported here should be further investigated if we are to generate a more comprehensive model of the neurology of PTSD, from acquisition to remission.

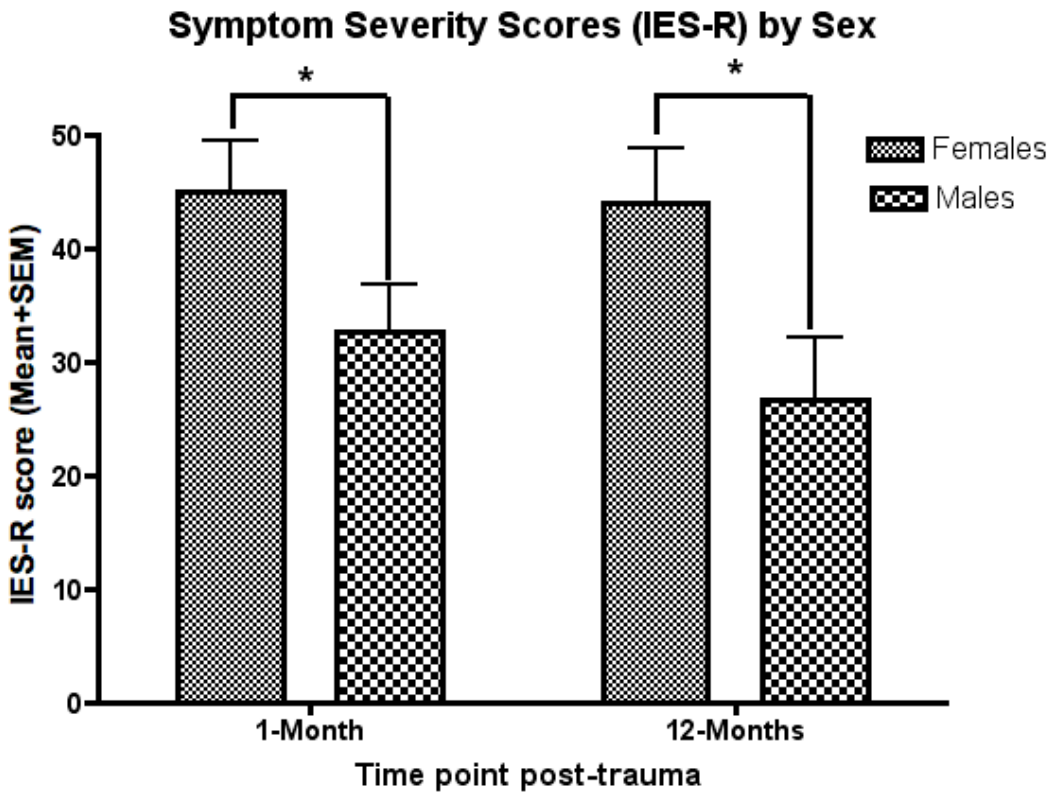
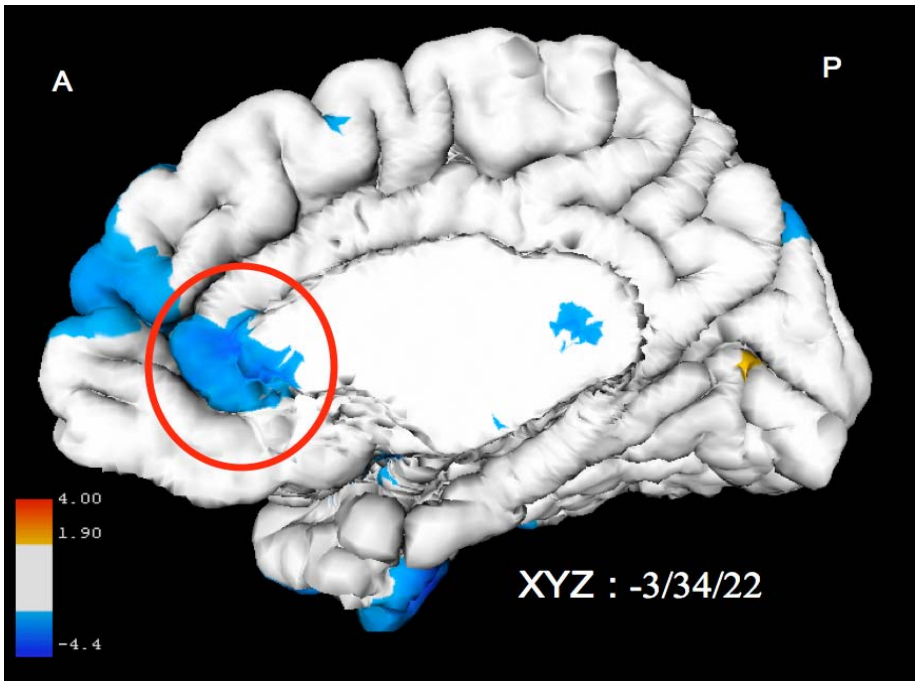


Figure 20. Impact of event-scale scores in males and females at one-month and twelve months post-trauma.

Male subjects show consistently less severe self-reported symptoms compared to females. Stars represent *p.*< .05.

(A)



(B)

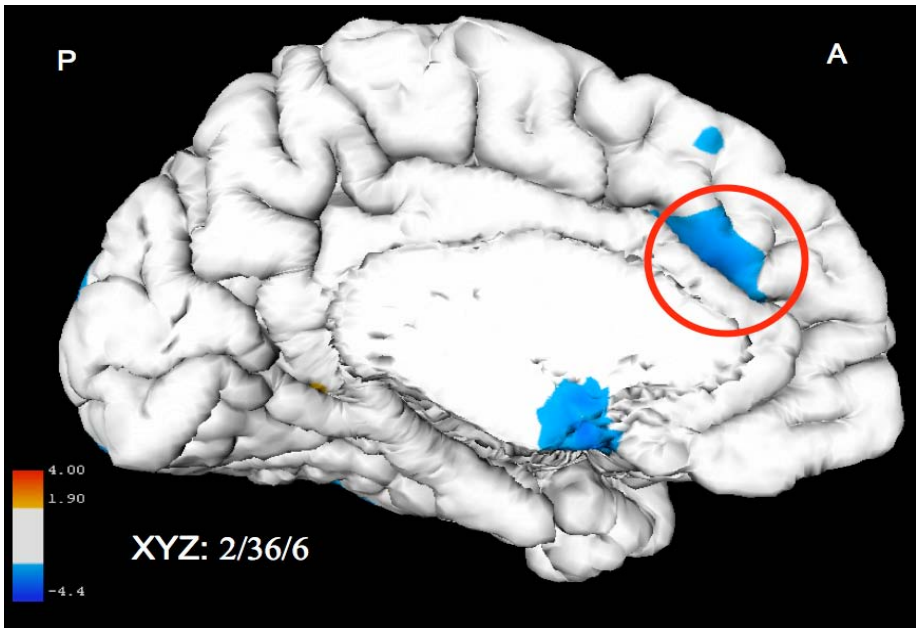


Figure 21. Correlations Cortical Thickness and Symptom Severity in the right and left ACC

Significant negative correlation between cortical thickness and self-reported symptoms severity (IES-R) in the (A) right pregenual [*t* (37) =

-3.42, *p.*< .003; XYZ MNI coordinates: -3/34/22] and (B) left dorsal [*t* (37) = -3.29, *p.*< .003; XYZ MNI coordinates: 2/36/6] anterior cingulate cortex across all subjects.

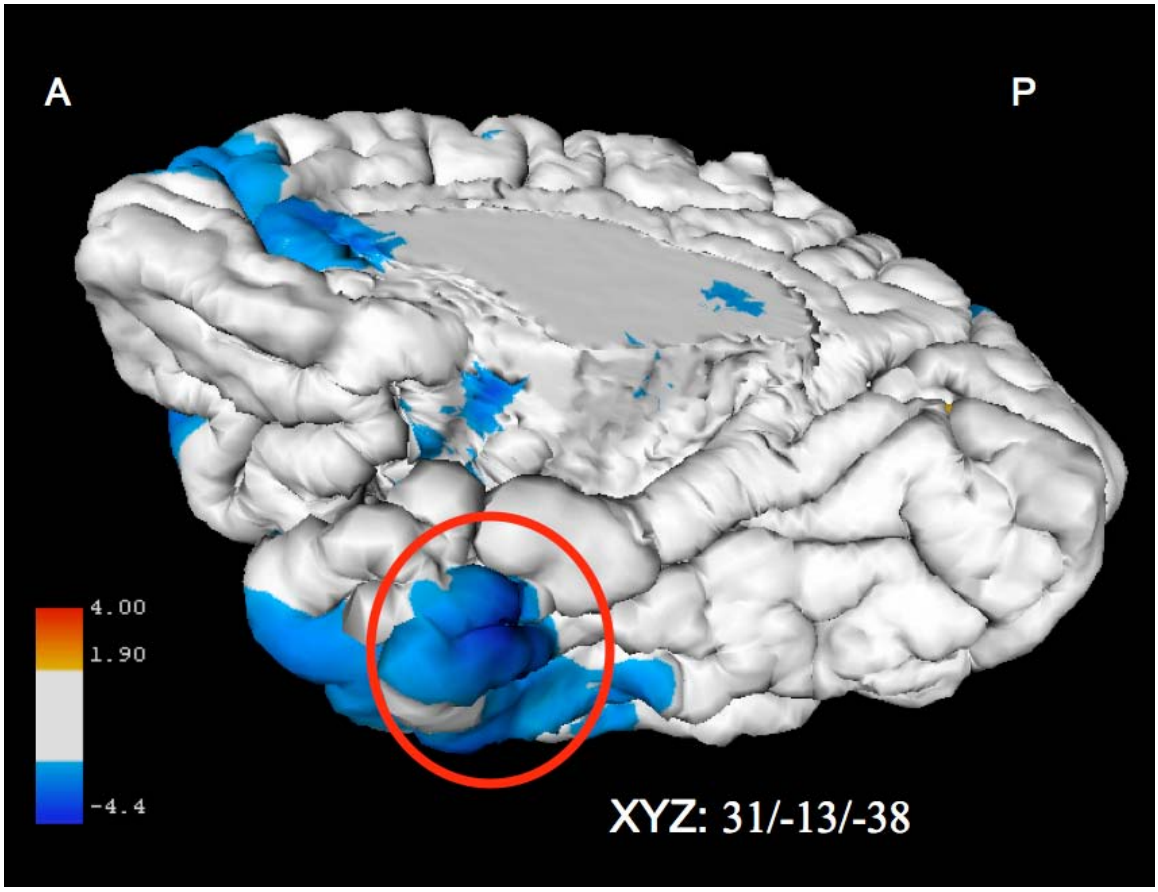


Figure 22. Correlation Cortical Thickness and Symptom Severity in Right Ventral Temporal Cortex

Significant negative correlation between cortical thickness and self-reported symptoms severity (IES-R) in the right ventral temporal cortex [*t* (37) = -4.31, *p.*< .000; XYZ MNI coordinates: 31/-13/-38].

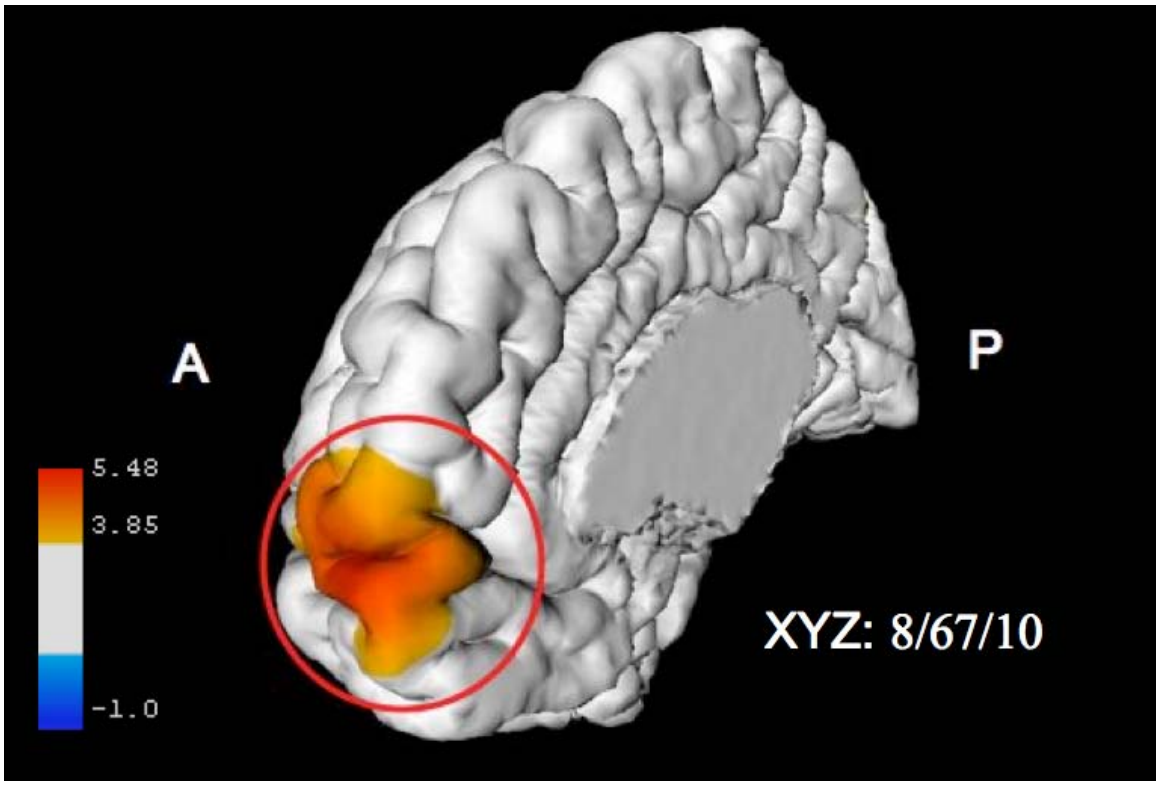


Figure 23. Correlation Cortical Thickness and Symptom Severity in Right Frontal Pole

Significant positive correlation between cortical thickness and clinician evaluated severity of symptoms (CAPS) in the right frontal pole [*t* (37) = 5.45, *p.*< .000; XYZ MNI coordinates: 8/67/10].

	Mean	SEM	Min	Max
Age	28.62	1.25	18	48
CAPS-T1	52.29	3.64	14	93
IES-R-T1	39.45	3.21	6	76
BDI-T1	6.86	0.93	0	21
CAPS-T2	27.55	3.65	0	91
IES-R-T2	36.74	3.84	0	84
BDI-T2	4.38	0.79	0	18

Table 4. Socio-demographic scores across all subjects

Displayed are means, standard error of the mean, minimal and maximal values; CAPS = Clinician Administered PTSD Scale; IES-R = Impact of Event Scale; BDI = Beck Depression Inventory; T1 = one month post-trauma; T2 = twelve months post-trauma.

	CAPS (T1)		IES-R (T1)		CAPS (T2)		IES-R (T2)	
	R	*p.*	*R*	*p.*	*R*	*p.*	*R*	*p.*
BDI T1	.768	.000	.523	.000	.396	.012	.410	.011
BDI T2	.453	.004	.716	.000	.477	.002	.498	.002

Table 5. Correlations between clinical scores across all subjects

CAPS = Clinician Administered PTSD Scale; IES-R = Impact of Event Scale; BDI = Beck Depression Inventory; T1 = one month post-trauma; T2 = twelve months post-trauma.

Chapter 6: DISCUSSION

1. Summary of Findings: Results and Limitations

The current thesis had two parallel objectives falling under a common concept: the interaction between fear and stress. In the first study, stress reactivity was assessed following a fear-conditioning task. While our task was not successful in inducing a significant rise in GSR across all subjects, a subset of our sample did show a significant conditioning. Interestingly, there was no difference in levels of cortisol to differentiate those who responded versus those who did not respond. Furthermore, there was no rise in cortisol in response to the task. Last, correlations were found between harm-avoidance and reward-dependence scales of the TPQ and cortisol levels both after the fear-conditioning task and in response to awakening. These traits did not correlate with the levels of GSR reactivity. When re-tested 72 hours after conditioning, subjects did not show any trace of conditioning.

In our second study, subjects were also asked to undergo a fear-conditioning paradigm, followed by a social stress task and immediate extinction. Despite a change in fear-conditioning paradigm, only 40% of the subjects of this study showed an increase in reactivity to the CS+. When comparing only the subjects that had shown the increase to the CS+, stress had a differential effect on immediate extinction learning. Specifically, a rise in cortisol levels induced by the TSST was associated with faster decline of the GSR to the CS+ during extinction. Using the Spielberger's Anxiety scale, we observed that anxiety levels were associated with the cortisol reactivity to the TSST but was not

associated with GSR. Last, in female subjects only, we observed an association between childhood adversity and the CAR.

Our third study left the field of pure fear-conditioning in healthy adults to investigate the levels of the stress hormone in PTSD, a disorder studied using the fear-conditioning model. Our results showed that the CAR was significantly higher in individuals who never developed significant PTSD symptoms following trauma exposure, compared with individuals who developed chronic PTSD and individuals who remitted from PTSD. Interestingly, this difference in CAR was true both one month and twelve months post-trauma, suggesting stability in time of the CAR.

Finally, our fourth study examined the cortical thickness of individuals exposed to a traumatic event both at one month and twelve months post-trauma. While we found significant negative correlations between the ACC and symptoms severity one month post-trauma, no association appeared at the twelve-months assessment. Furthermore, at the one-month assessment, we detected significant associations between frontal pole as well as ventral temporal area and symptoms severity. This association was again not found at the follow-up assessment.

The common limitation shared by all our studies was the relatively small number of participants. Our first study was a pilot project that included only 19 subjects in a correlational design. Our fear-conditioning paradigm was successful for only 30% of

participants, a rate that was improved slightly to 40% in our second study. In this latter case, because of pre-task random attribution to the Stress vs. Control group, we were unable to balance the responders and non-responders to the fear-conditioning acquisition. Therefore, we only had 6 responders who completed the stress task. Despite this low number, we did find a significant difference in the reactivity to the CS+. The question remains as to the generalization of this finding, which is undermined by the small sample size. Other studies that have used fear-conditioning paradigms have generally used samples of equivalent size (total of around 40 subjects). As we mentioned in the discussion section of each project, there are multiple factors that may have contributed to this general lack in response. One important factor may have been the nature of the US. Loud bursts of high pitch tones have been used successfully in other studies. However, it would appear that our tone might not have been aversive enough to provoke significant changes in GSR. Even though we asked participants to adjust the volume to their personal maximal level of tolerance, it may not have been enough. In our first study, it is possible that the US occurred not often enough to create significant conditioning. In our second study, despite higher absolute numbers of US and a different, biphasic contingency rate, the tone did not induce clear association with the CS+ in terms of GSR. The subjects that were identified as Responders further showed greater GSR only at the third quarter in the acquisition phase. Therefore, the US and its parameters may have played a key role in the low rate of success of our tasks.

Other parameters in our tasks may also have played a role in this rate of acquisition. We chose colors as a CS based on previous studies that showed significant acquisition. We also used pictures drawn from the IAPS data set based on Ohman's bio-preparedness theory (Ohman & Mineka, 2001), in order to facilitate discrimination between the CS+ and CS-. However, it is possible that colors were not salient enough or that the pictures induced distraction. Contrasted with other studies where subjects view the stimuli passively, we also asked participants to actively judge between pictures during the tasks. This was done to increase involvement in the task. We can hypothesize that this basic judgment task distracted subjects from the fear-conditioning aspect. In sum, a weaker US combined with a CS that was not sufficiently salient might have lost the competition for attention to the "other" task of judging pictures.

Another common factor to both our studies was that we included all GSR data in our analyses. As we mentioned in the introduction to this thesis, the protocols available to analyze GSR vary greatly between studies. Many of these protocols chose certain criteria for inclusion of a response, e.g. values above 0 μS (Vervliet, et al., 2005), 0.01 μS (Brignell & Curran, 2006), 0.02 μS (Zorawski, et al., 2006; Zorawski, et al., 2005), or outside a specific time delay (Jackson, et al., 2006). The only criterion we used was exclusion of outliers. This may have contributed to the global absence of conditioning. Studies that code negative GSR as 0 skew the distribution of their data towards the positive end of the spectrum. By including all data, we allowed for greater variance to emerge, thus diminishing our capacity

to detect between-group differences. However, we feel that this inclusion of all data allowed for the interesting Responders/Non-responders pattern to emerge. This was a choice that we made in the hopes of characterizing better the interaction between varying levels of cortisol, personality traits and GSR.

Our third and fourth studies also suffered from small samples, though in this case it may be explained by the relative difficulty present in finding individuals exposed to a recent traumatic event that are willing to get involved in research projects. In our clinical sample, we did have more individuals exposed to motor-vehicle and work-related accidents, since these victims tend to present themselves at emergency rooms more than victims of assault, either physical or sexual. Furthermore, in the study on the CAR, we did have uneven distribution of sex across groups. This might limit the generalization of our findings across all individuals suffering from PTSD, males or females. Furthermore, in the CAR study, we did not control for use of oral contraceptives or the phase of the menstrual cycle in our female subjects, which might have influenced the levels of cortisol secreted (Kirschbaum, et al., 1999; Kudielka & Wust, 2010; Zorawski, et al., 2006).

Despite these limitations, the studies composing this thesis draw an interesting portrait of the interaction between fear and stress, both in a healthy sample as well as in individuals suffering from PTSD.

2. On Fear-Conditioning: Contributions to the Learning of Fear

Our first studies have shown that fear-conditioning, while being a useful paradigm, still presents some difficulties that should be addressed. First, while the choice of stimuli do not matter much in animal research, it appears that human studies may be more complicated than as been highlighted by previous studies.

One of the theoretical hallmarks of fear-conditioning is the capacity to create a CR by using any stimuli, especially the CS. This raises the issue of the relevance of the results observed for modeling real-world situations and fearful behavior. In the current study, we used artificial stimuli such as color combined with pictures of snakes. Based on the theory of biopreparedness (Ohman & Mineka, 2001), this specific type of stimulus should have contrasted enough with the neutral pictures chosen for the CS- and therefore resulted in greater acquisition of the CR to the CS+. Our results do not support this theory. One possible explanation for this is that the pictures were not fearful enough. The images were selected from the International Affective Picture System (IAPS) and rated by various volunteers. From this pool, the ten pictures that were judged the scariest were selected to be included in the task. This rating was done on a single observation of the picture while the task asked subjects to view the pictures multiple times. It is thus possible that participants habituated quickly to the pictures, therefore decreasing the intrinsic arousing property of the images. Additionally, the nature of the CS was determined by the color of the screen. While the contingencies were

explicitly stated to participants, the differences in colors may not have been salient enough for participants to generate a reaction to the CS+ alone. This lack of discrimination between CSs indicates that color and pictures were not the best predictors of the occurrence of the US. In the case of the second study, since the task was based on a constant inter-stimuli interval, it is possible that the timing became more predictive of the possible occurrence of the US in most subjects, whereas only the responders identified the threat with the color of the screen. Therefore, we may attribute to both the lack of saliency and regular time interval the relatively weak performance of our fear-conditioning paradigms in eliciting reliable CR across all subjects.

The other consideration to address is the nature of the US used in studies. Similarly to other studies, we used a burst of loud tone in the high frequencies. In order to minimize potential habituation, we further varied the exact noise by changing the frequency. However, based on the low percentage of subjects who showed an increased reactivity to the CS+, it may be that such a US is not sufficient to create significant fear in most subjects. One potential reason for this performance of the US may be the target sensory system. It is possible that targeting the auditory system may have led to faster habituation, despite a relatively low rate of pairings. Individuals are repeatedly exposed to noises in daily life, especially younger people. For example, the sound of jackhammers, quite frequent in an urban setting, approximates a sound level of 100dB, which is the most often used volume for the US. Therefore, we propose that frequent exposure to loud noise in daily life may reduce the ability of the noise to remain

novel and threatening. In the case of our studies, the rapid habituation to the US itself could have led to the CS+ not acquiring the aversive quality necessary for the emergence of a discriminative CR.

As we mentioned in the introduction, most studies in human fear-conditioning have used mildly painful electrical shock as US. Contrasting with our studies, these studies generally report reliable acquisition across all subjects with groups of similar size compared to our samples. Interestingly, the level of reactivity to the CS+ does not differ from the levels observed in our study amongst the responders. It would therefore seem that shocks are not intrinsically more aversive than a loud noise, since the actual CR is not significantly different. What would differ is the percentage of people that react to a shock compared to a noise or the percentage of individuals that fail to habituate to this type of stimulation. In other words, shocks would not create a stronger CR but rather would induce a CR in more people compared to other US. Furthermore, studies that have investigated long-term memory of fear-conditioning have used shocks for US (M. R. Milad, et al., 2006; Zorawski, et al., 2006) and a 24 hours delay to examine extinction learning. Contrasting with our first study where we did not detect any memory of the CR 72 hours after initial conditioning, these studies observed the presence of a significant CR. We can therefore conclude that, while loud noises may induce significant conditioning in some subjects, shocks may prove more useful to study long-term effects of fear-conditioning. It remains to be seen how long exactly the CR can last before a lack of repetition induces natural forgetting.

One interesting finding that emerges from our two fear-conditioning studies is the presence of a certain percentage of individuals that responded to the acquisition of fear. This phenomenon has also been reported by Stark and colleagues (Stark, et al., 2006); they found that in their female subjects, only 11-13% of subjects displayed a significantly greater response to the CS+ compared to the CS-. In their male subjects of the cortisol condition, none responded significantly more to the CS+. Contrary to our study, these authors used electrical shocks as US. This partial success in conditioning raises the question of what happened in the other percentage of individuals who were exposed to the same procedure. While we cannot conclude from a negative finding, we must address this issue, since it might help model better the relatively low proportion of individuals who develop PTSD after exposure to a traumatic event. One recent paper by Bush and colleagues (D. E. Bush, et al., 2007) has focused on the existence of various phenotypes in reactivity to fear in genetically identical rats. Selecting subjects that showed either low or high percentage freezing in one fear-conditioning paradigms, the authors illustrated that the high reactive rats showed greater reactivity to the CS+ 48 hours and 72 hours after conditioning. In a second study, these authors trained rats in extinction and selected subjects based on their percentage freezing response in extinction. One day later, in an extinction retention test, rats of the high freezing group showed greater retention of extinction. The crucial aspect of this report is that all subjects were genetically identical and were raised in similar environments. No specific manipulations were conducted to induce

differences in reactivity to the conditioning task or extinction learning. As the authors mention in their discussions, many studies have investigated between-groups differences. However, few studies have examined the differences in reactivity to fear in itself. Since this was not the primary objective of our two studies, we cannot draw firm conclusions. Our analyses did not reveal significant differences in terms of cortisol or trait anxiety between responders to the task and non-responders, which leads us to posit that GSR reactivity may not be linked to personality traits of anxiety or to the HPA-axis. Furthermore, based on the success rate of our fear-conditioning paradigms, the use of a mildly aversive US such as a loud noise may prove more useful in identifying possible phenotypes in fear reactivity. Also based on our results, the inclusion of various points of measure is essential if one focuses on quantifying the rate of acquisition of the CR across subjects.

3. Of Fear and Stress

The results from the present thesis help shed new light on the interaction between the SNS and the HPA-axis. Specifically, our first project illustrates how fear-conditioning in itself may not be novel, uncontrollable and threatening enough to induce a significant response of the HPA-axis despite the presence of a significant activation of GSR. Our replication of the decline in cortisol levels observed by Zorawski and colleagues (Zorawski, et al., 2006; Zorawski, et al., 2005) lead us to conclude that the HPA-axis might need stronger stimulation to trigger a cortisol secretion. Since cortisol is an important modulator

of learning and memory, it is surprising how few studies have taken it into account. As we reviewed in the introduction, most studies have investigated the interaction between stress and verbal declarative memory and have found the predicted increase in memory, especially for emotional material. However, the effects of stress at the level of the periphery may be very different. Indeed, our second study was the first to investigate the effect of post-learning stress induction on immediate extinction, contrasting with the work of Zorawski and colleagues (Zorawski, et al., 2006), who investigated the effects 24 hours after acquisition. Contrasting with the verbal declarative performances, these authors did not find any modulation of conditioning by cortisol at the extinction test, despite splitting their sample with a median-based split. Our results are the first to indicate a role of cortisol on the CR in the immediate aftermath of acquisition. This negative relation between cortisol and GSR has been shown to be present even at the acquisition stage (Merz, et al., 2010). In a recent study published by Tabbert and colleagues (Tabbert, et al., 2010), the authors have shown that increased cortisol levels were associated with an increase in the activity of the amygdala and ACC to the CS- relative to the CS+. Although this effect, did not translate at the level of GSR, this finding supports our view that cortisol may actually interfere with the CR and enhance the acquisition of extinction learning. Interestingly, we must note that Tabbert and colleagues tested only women who were using oral contraceptives, which is similar to our own study design, supporting the observation of an influence of gonadal hormones on fear-conditioning.

The convergence of evidence of the potentiating effect of cortisol on extinction learning at the level of the periphery has opened the field to new therapeutic approaches that target disorders where extinction learning may be insufficient or inefficient. Two recently published reviews have highlighted the emergence of glucocorticoid administration as a novel way to enhance the efficiency of exposure therapy for anxiety disorders such as PTSD (Bentz, Michael, de Quervain, & Wilhelm, 2010; Yehuda, 2009; Yehuda & Golier, 2009). While the exact mechanism of action of cortisol on the pathophysiology of these various disorders is not completely understood, the results from our studies speak in favor of exploring this new field with well-controlled studies.

One last issue that we wish to discuss refers to the correlations between personality variables and biological measures. It is interesting that in both studies, there was an absence of correlation between personality traits, whether based on the TPQ or the STAI, with GSR during the tasks. Conversely, we did observe significant correlations between cortisol secretion and personality. Furthermore, we observed in females a link between childhood adversity and cortisol awakening response. In previous studies, anxious personality trait has been linked with reactivity to fear-conditioning (Barrett & Armony, 2006; Otto, et al., 2007). However, we did not replicate this in our studies. It is possible that our sample size was not sufficient to detect a subtle effect. Interestingly though, our sample size was comparable to other studies and was sufficient to detect correlations with cortisol. Our data lead us to think that personality factors may emerge at a later

stage of development compared to the reactivity of the SNS. While we do not deny the importance of early life adversity on the development of the nervous system, it is possible that it affects structures linked with extinction learning rather than the amygdala itself. Data from our team has already shown the importance of parental bonding in the development of the hippocampus and stress reactivity (Buss, et al., 2007; Engert, Efanov, Dedovic, Dagher, et al., 2010). But as we have shown before, stress reactivity does not correlate with GSR activity to fear. Therefore, it is possible that sensitivity of the fear response may not be influenced by development during childhood, but rather that regulation of this response through central nervous system input as well as endocrine modulation may be the target of developmental influences.

4. Of PTSD: Revisiting a Tale of Fear and Stress

One question we asked in this thesis concerns the use of fear-conditioning as a model for PTSD. It must be recalled that in the case of PTSD, the model (R. K. Pitman, 1989) predicts clearly that the traumatic event induces fear conditioning that will be over-consolidated due to the massive secretion of stress hormones. This would consequently lead to the cued and un-cued reappearance of the traumatic memories, the avoidance of reminders as well as the general state of hyperarousal. In view of our results, we must address certain points in the model.

The first prediction of the model is that the fear-conditioning occurring at the time of trauma will create an increase in levels of circulating cortisol that will support the over-consolidation. However, our first study clearly indicated that a simple fear-conditioning paradigm does not induce a change in the levels of circulating cortisol as would be predicted by the model. This suggests that the data acquired so far lacks an important dimension that may contribute significantly to our understanding of the etiology of the disorder from healthy volunteers. Our second study aimed at filling this gap and revealed an unexpected effect of cortisol on the GSR: whereas most studies predicted that cortisol would increase the activity of the SNS, we observed a potentiation of extinction and decline in SNS. This finding goes against the original model of PTSD but seems to better reflect the current state of the literature on cortisol in PTSD as we mentioned in the introduction (Yehuda, 2009). Recently published studies of the influence of cortisol on the brain areas involved in fear-conditioning have highlighted the differential effect of the hormone on the key structures (Merz, et al., 2010; Tabbert, et al., 2010).

Considering the differential effect we observed in males and females with respect to GSR and cortisol reactivity in our second study, we propose that studies using fear-conditioning to model PTSD must include hormonal levels and controls for gonadal hormones in order to better understand the sexual dimorphism we observed in our samples. Many studies have found stronger GSR response in males (Jackson, et al., 2006; M. R. Milad, et al., 2006; Zorawski, et al., 2006). However, epidemiological studies show that more women than men

develop PTSD upon exposure (Breslau, et al., 1998), something we replicated in our studies by highlighting both a greater reactivity to fear in women as well as a greater proportion of women in our clinical samples. Considering the interaction between gonadal hormones, cortisol as well as consolidation of fear acquisition and extinction, our results indicate that the current model to study PTSD based on simple fear-conditioning in a vacuum may not be sufficient to explain the emergence of symptoms, lack of extinction and account for the sexual dimorphism observed in clinical settings.

The results from our third and fourth study also indicate that simple fear-conditioning may not fully account for the spectrum of symptoms of PTSD. The results from our study on the CAR in civilian traumas indicate that the HPA-axis dysregulation may account for the maintenance of the symptoms as early as one month post-trauma. This blunted CAR could be seen as a risk factor for the incapacity to learn and consolidate extinction after the trauma. This interpretation does fit with the findings of our second study where cortisol increased the rate of extinction learning. Also, it has been shown that the HPA-axis possesses a critical period of development during childhood (Buss, et al., 2007; Engert, Efanov, Dedovic, Dagher, et al., 2010; Engert, Efanov, Dedovic, Duchesne, et al., 2010; Meaney, 2001; Zhang, et al., 2006). Our data point in this direction and presents another important factor in understanding the alterations observed in PTSD. If the HPA-axis is involved in suppressing the expression of the CR and therefore of symptoms of PTSD, early life adversity is potentially a key factor leading to greater risk to develop chronic PTSD upon exposure

especially in females. In sum, our findings suggest that a complete model of PTSD should include not only fear but also cortisol levels both from a reactive as well as a basal perspective. In addition to this, the model should include a strong developmental perspective assessing past abuse and/or neglect in order to better characterize the higher risk trajectories of development.

Our last study adds an interesting dimension to studies of the neurology of PTSD in civilian trauma. In the introduction, we reviewed the key structures involved in acquisition and extinction of fear. Confirming the predictions of the fear-conditioning model (R.K. Pitman, et al., 2001), we did observe a negative correlation between the thickness of the ACC and severity of symptoms. Our findings also identified two novel structures that have not been targets of functional studies yet, since they are not components of the typical fear axis identified in the animal literature (J. E. LeDoux, 2000). The presence of an association between the ventral temporal area as well as the anterior frontal pole with symptoms severity suggest that, while fear-conditioning may explain the acquisition of the disorder at the time of trauma and in the immediate aftermath, the emergence of all three clusters of symptoms may not be fully accounted for by the fear axis. The social aspect of the traumatic exposure as well as the cognitive reprocessing following the event are two important factors that may determine successful adaptation to the impact of trauma. These two processes are significantly associated with the structures that were detected in our last study.

Considering the results from our first two studies in relationship to PTSD and the data from our third and fourth studies, we must question the validity of a laboratory-based fear-conditioning paradigm as a model for the experience of trauma. The traumatic experience is of such intensity that it might be impossible to replicate in a safe and ethical context. It is true that studies of fear-conditioning do provoke responses from the same structures of the central nervous system that are associated with PTSD. However, the magnitude of those reactions might differ substantially. The experience of trauma may also engage a variety of responses that cannot be triggered by a simple fear-conditioning paradigm. Future studies may have to look for newer methods to better replicate the variety and intensity of reactions to the traumatic experience. Furthermore, as we mentioned in the discussion of chapter 5, other emotions are involved in PTSD, such as shame and guilt. Fear-conditioning is not a paradigm well-adapted to study these emotions. Future studies may have to create new paradigms that are better suited to study this variety of emotions triggered by one single event.

5. Concluding Remarks

The aim of this thesis was two-fold but falls under the greater objective of investigating the interaction between fear and stress. Learning what and when to fear is an essential feature across all animal species to increase adaptation to various circumstances and potential threats. However, learning what and when *not* to fear is as essential to promote adaptive behavior, since feeding and reproductive

behavior depend on the parasympathetic nervous system, which is inhibited by activation of the sympathetic nervous system. In order to fully understand fear, it is therefore mandatory to study both learnings in order to appreciate fully the spectrum of inter-individual differences and adaptability.

Furthermore, this thesis shows the importance of integrating endocrine measures when investigating emotional learning. The HPA-axis has been recognized as a potent modulator of learning in a variety of tasks. It therefore came as a surprise to see the relative paucity of studies that attempted at manipulating endogenous cortisol levels in investigations of a well-known field such as fear conditioning and extinction. The expected co-activation of the sympathetic and endocrine systems did not appear in our studies, underlying the great care that must be taken when translating data from the animal studies to human studies. In addition, the differential effect of cortisol on autonomic measures and verbal memory adds a new dimension to the field of cognitive neurosciences in that it highlights the relative independence of these two systems that have been shown to potentially contribute to the emergence of various disorders such as PTSD.

This thesis also highlights the importance to study PTSD with a more comprehensive approach. The original model proposed by Pitman (R. K. Pitman, 1989; R.K. Pitman, et al., 2001) does provide a sound approach to the peritraumatic experience. The temptation to study fear-conditioning without measures of endocrine markers and apply

these findings to PTSD research and treatment might be strong. However, it's a trap to believe that fear and explicit memories of fear can account for the three clusters of symptoms that equally contribute to the suffering of individuals with PTSD. Social behavior, stress, developmental experiences and cognitions must be integrated into the equation if we are to understand the resilience factors and dynamic changes brought on by the traumatic exposure that, in a percentage of individuals at risk, will result in the chronic presence of symptoms. This integration of multiple agents, based on comprehensive studies in healthy population, might then be exported to the clinical setting in a specific combination of pharmacological, psychological and social interventions for victims of trauma. This could maximize their chances of remitting and remembering what and when not to fear.

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