

Universität Trier - Fachbereich I - Psychobiologie

Dissertation zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)



Stress and Aggression - a psychophysiological approach

Autor:

Robina Böhnke, Dipl.-Psych.

Eingereicht im Mai 2010

Gutachter:

Dr. rer. nat. Ewald Naumann

Prof. Dr. med. Hartmut Schächinger

Dissertationsort: Trier

Tag der mündlichen Prüfung: 07.07.2010

This dissertation thesis and the presented research were performed at the

Psychophysiological Laboratory
Department of Psychology - University of Trier

Affiliation of the Supervisors:

Dr. rer. nat. Ewald Naumann
Psychophysiological Laboratory - Department of Psychology
University of Trier

Prof. Dr. med. Hartmut Schächinger
Division of Clinical Physiology - Institute of Psychobiology
University of Trier

Funded by the German Research Foundation (Deutsche
Forschungsgemeinschaft: DFG), Project GRK 1389/1:

International Research Training Group
**"Psychoneuroendocrinology of Stress: From Molecules and Genes to Affect
and Cognition"**

Project H: "On the relationship of stress and aggression"

Acknowledgments

First, I want to thank a long list of people, who directly or indirectly contributed to this work.

Without you, it would not have been possible:

To Ewald, for giving me this wonderful opportunity in the first place, and giving me great opportunities before, and for your support and encouragement that helped me get through it.

To Katja, for a good collaboration over the years and being a companion in foreign countries and continents.

To Menno, for inspiring this research project and always being there with great advice and stimulating thoughts. Thanks for your welcome in Leiden and your help in getting us connected with people in the field.

To all in the Psychophysiological Laboratory:

To Renate, for your great help in getting the research through and for being a wonderful and supporting colleague.

To Helmut, for your technical support concerning the data acquisition and everything else.

We miss you.

To Michael and Patrick, for giving me a sneak peak into the world of science, and for all our great talks and advice that you gave me along the way.

To Florian, for being a good colleague and for helping me with my papers.

To all of the research students at the lab: Lisa, Ulf (I miss you two), Leander, Lea, Juliane, and Felix. Thank you for your hard work and your commitment and discipline. Without you, the data would not have been half as good. To Johann and Thomas, for your great skills with EPrime and making these complicated experiments possible. Thanks for your hard work and for your energy that you put into every project.

To Hartmut and his entire work group, especially André and Steffen, for your support with the experiments and papers. For all of you, for some great times at conferences, summer schools, and workshops.

To the rest of the Leiden team of the IRTG, especially Melly and Ron, for opening my eyes to completely new ideas, thoughts and research fields and for very helpful comments on our designs.

To Julia and Angelika, for being so dedicated and motivated and for the great fun times we had. I can now part from this project knowing that it is in your very capable hands.

To the family additions that I gained during that time: To Gundula, Peter and Gesche. Thank you for making a perfect find even better.

To my mom, and my bros, for always supporting me and lightening up my life.

To the rest of the family clan around the world, especially the ones in England and Michigan.

And most importantly, to mine...my own...my precious. You came to me, and once you took hold of me you never let go. You complete me.

General Abstract

Aggression is one of the most researched topics in psychology. This is understandable, since aggression behavior does a lot of harm to individuals and groups. A lot is known already about the biology of aggression, but one system that seems to be of vital importance in animals has largely been overlooked: the hypothalamic-pituitary-adrenal (HPA) axis. Menno Kruk and József Haller and their research teams developed rodent models of adaptive, normal, and abnormal aggressive behavior. They found the acute HPA axis (re)activity, but also chronic basal levels to be causally relevant in the elicitation and escalation of aggressive behavior. As a mediating variable, changes in the processing of relevant social information is proposed, although this could not be tested in animals. In humans, not a lot of research has been done, but there is evidence for both the association between acute and basal cortisol levels in (abnormal) aggression. However, not many of these studies have been experimental of nature.

Our aim was to add to the understanding of both basal chronic levels of HPA axis activity, as well as acute levels in the formation of aggressive behavior. Therefore, we did two experiments, both with healthy student samples. In both studies we induced aggression with a well validated paradigm from social psychology: the Taylor Aggression Paradigm. Half of the subjects, however, only went through a non-provoking control condition. We measured trait basal levels of HPA axis activity on three days prior. We took several cortisol samples before, during, and after the task. After the induction of aggression, we measured the behavioral and electrophysiological brain response to relevant social stimuli, i.e., emotional facial expressions embedded in an emotional Stroop task. In the second study, we pharmacologically manipulated cortisol levels 60min before the beginning of the experiment. To do that, half of the subjects were administered 20mg of hydrocortisone, which elevates circulating cortisol levels (cortisol group), the other half was administered a placebo (placebo group).

Results showed that acute HPA axis activity is indeed relevant for aggressive behavior. We found in Study 1 a difference in cortisol levels after the aggression induction in the provoked group compared to the non-provoked group (i.e., a heightened reactivity of the HPA axis). However, this could not be replicated in Study 2. Furthermore, the pharmacological elevation of cortisol levels led to an increase in aggressive behavior in women compared to the placebo

group. There were no effects in men, so that while men were significantly more aggressive than women in the placebo group, they were equally aggressive in the cortisol group. Furthermore, there was an interaction of cortisol treatment with block of the Taylor Aggression Paradigm, in that the cortisol group was significantly more aggressive in the third block of the task. Concerning basal HPA axis activity, we found an effect on aggressive behavior in both studies, albeit more consistently in women and in the provoked and non-provoked groups. However, the effect was not apparent in the cortisol group. After the aggressive encounter, information processing patterns were changed in the provoked compared to the non-provoked group for all facial expressions, especially anger.

These results indicate that the HPA axis plays an important role in the formation of aggressive behavior in humans, as well. Importantly, different changes within the system, be it basal or acute, are associated with the same outcome in this task. More studies are needed, however, to better understand the role that each plays in different kinds of aggressive behavior, and the role information processing plays as a possible mediating variable. This extensive knowledge is necessary for better behavioral interventions.

Zusammenfassung

Aggression ist eine der meist untersuchten Phänomene in der Psychologie. Das ist verständlich, da aggressives Verhalten Individuen und Gruppen sehr schadet. Vieles ist schon bekannt über die biologischen Korrelate der Aggression, aber ein System, welches in Tieren sehr wichtig zu sein scheint, wurde bisher vernachlässigt: die Hypothalamus-Hypophysen-Nebennierenrinden-Achse (HHNA, in englisch HPA). Menno Kruk und József Haller und deren Forschungsgruppen entwickelten anhand von Ratten Modelle für adaptives normales, aber auch für abnormales aggressives Verhalten. Sie fanden, dass akuter HHNA Aktivität, wie auch dem chronischen Ruhespiegel eine kausale Rolle bei der Entstehung und Eskalierung von aggressivem Verhalten zukommt. Als eine mediierende Variable nahmen sie Veränderungen in der Verarbeitung von relevanten sozialen Informationen an, obwohl sie dies nicht in Ratten testen konnten. In Menschen gab es zu dem Thema bisher nicht viele Studien, aber auch im Menschen gibt es Befunde für den Zusammenhang zwischen akuten und basalen Cortisolspiegeln und (abnormaler) Aggression. Allerdings gab es bisher wenige experimentelle Studien.

Unser Ziel war es, das Verständnis für die Rolle, die basale chronische HHNA Aktivität, wie auch der akute Cortisolspiegel bei der Entstehung von aggressivem Verhalten spielen, zu erweitern. Wir führten deshalb zwei Studien durch, beide mit gesunden studentischen Stichproben. In beiden Studien induzierten wir aggressives Verhalten mit einem gut validierten Paradigma aus der Sozialpsychologie: dem Taylor Aggressionsparadigma. Die Hälfte der Probanden durchlief allerdings eine nicht provozierende Kontrollbedingung. Wir nahmen stabile basale Maße der HHNA Aktivität an drei Tagen vor dem Experiment. Zudem erhoben wir zahlreiche Cortisolproben vor, während, und nach der Aufgabe. Nach der Aggressionsinduktion erfassten wir die Reaktionen auf relevante soziale Stimuli, in diesem Fall emotionale Gesichtsausdrücke, die in einem emotional Strooptest eingebettet waren, sowohl auf Verhaltens-, als auch auf elektrophysiologischer Ebene. In der zweiten Studie manipulierten wir darüber hinaus pharmakologisch 60 Minuten vor Start des Experimentes die Cortisolspiegel unserer Probanden. Hierfür verabreichten wir der Hälfte der Probanden 20mg Hydrocortison, welches die zirkulierenden Cortisolspiegel angeht (Cortisolgruppe), die andere Hälfte bekam ein Placebo (Placebogruppe).

Ergebnisse konnten zeigen das akute HHNA Aktivität tatsächlich relevant für aggressives Verhalten ist. In der ersten Studie fanden wir einen Unterschied zwischen den provozierten und nicht provozierten Probanden in den Cortisolspiegeln nach der Aggressionsinduktion (also höhere Reaktivität der HHNA). Dieser Effekt konnte allerdings in Studie 2 nicht repliziert werden. Außerdem verstärkte die pharmakologische Erhöhung der Cortisolspiegel, verglichen mit der Placebogruppe, das aggressive Verhalten in Frauen. Bei den Männern fanden sich keine Effekte. Während Männer signifikant aggressiver waren als Frauen in der Placebogruppe, war dieser Geschlechtseffekt in der Cortisolgruppe verschwunden. Außerdem gab eine Wechselwirkung zwischen der Cortisolmanipulation und den Blöcken des Taylor Aggressionsparadigmas: die Cortisolgruppe war signifikant aggressiver als die Placebogruppe im dritten Block der Aufgabe. Was die basale HHNA Aktivität angeht, so fanden wir in beiden Studien eine Assoziation mit aggressivem Verhalten, allerdings waren diese Effekte konsistenter in Frauen und sowohl in der provozierten, als auch in der unprovozierten Gruppe zu finden. Nur in der Cortisolgruppe fand sich dieser Zusammenhang nicht. Nach der aggressiven Auseinandersetzung waren die Muster der Informationsverarbeitung für die provozierte Gruppe im Vergleich zur nicht provozierten Gruppe für alle emotionalen Gesichtsausdrücke, besonders aber für Wut, verändert.

Diese Ergebnisse weisen auf die wichtige Rolle hin, die die HHNA Aktivität bei der Entstehung von aggressivem Verhalten bei Menschen spielt, hin. Besonders hervorzuheben ist, dass unterschiedliche Veränderungen dieses Systems, also basal oder akut, zu dem gleichen Ergebnis in dieser Aufgabe führen. Allerdings sind noch weitere Studien vonnöten, um noch besser zu verstehen, welche Rolle jede dieser Veränderungen bei unterschiedlichen Arten der Aggression spielt und um zu klären, welche Rolle Informationsverarbeitung dabei als mediierende Variable spielt. Dieses extensive Wissen ist nötig, um bessere Interventionen auf Verhaltensebene zu entwickeln.

Content

Acknowledgments.....	i
General Abstract.....	iii
Zusammenfassung.....	v
Index of Publications.....	x
I. The framework.....	1
1.1 Aggressive behavior.....	1
1.1.1 The General Aggression Model.....	1
1.1.2 biological theories.....	2
1.2. Stress & Aggression.....	3
1.2.1 Stress and the HPA axis.....	3
1.2.2 The results of Menno Kruk et al. concerning acute HPA axis activity.....	5
1.2.3 The results of Haller et al. concerning basal HPA axis activity.....	6
1.3 Stress and aggression - evidence in humans.....	7
1.3.1 Acute HPA axis activity.....	7
1.3.2 Basal HPA axis activity.....	8
1.3.3 Information processing.....	9
1.4 Research questions.....	9
1.5 Main findings and general discussion.....	11
1.5.1 Acute HPA axis activity and reactivity, and aggression.....	12
1.5.2 Basal HPA axis activity and aggression.....	12
1.5.3 Information processing and aggression.....	12
1.5.4 Discussion of the results in the context of the work of Menno Kruk et al.....	13
1.5.5 Discussion of the results in the context of the work of Haller et al....	14
1.5.6 Concerning information processing.....	14
1.6 A different perspective on things: the HPA axis as a dynamic, context dependent, and adaptive system.....	15
1.7 Further research ideas.....	17
1.8 Three notes from the psychologist within.....	19
1.9 Conclusion.....	20

II. The relationship between basal and acute HPA axis activity and aggressive behavior in adults	21
2.0 Abstract.....	22
2.1 Introduction	22
2.2 Methods	25
2.2.1 Subjects.....	25
2.2.2 The Taylor Aggression Paradigm.....	25
2.2.3 Acute HPA axis activity	26
2.2.4 Basal HPA axis activity.....	26
2.2.5 Procedure	27
2.2.6 Statistical analyses	28
2.3 Results	29
2.3.1 Subjects' characteristics	29
2.3.2 Manipulation check: aggressive behavior in the Taylor Aggression Paradigm.....	30
2.3.3 Basal cortisol levels and aggressive behavior	30
2.3.4 Acute cortisol levels and aggressive behavior.....	31
2.4 Discussion	32
2.i Acknowledgments	36
2.ii References	37
III. Exogenous cortisol enhances aggressive behavior in females, but not in males ...	42
3.0 Abstract.....	43
3.1 Introduction	43
3.2 Methods	46
3.2.1 Participants	46
3.2.2 The Taylor Aggression Paradigm.....	47
3.2.3 Feelings of anger and negative affect	48
3.2.4 Acute HPA axis activity	48
3.2.5 Basal HPA axis activity.....	49
3.2.6 Procedure	49
3.2.7 Statistical analyses	51
3.3 Results	52
3.3.1 Participants' characteristics and manipulation check.....	52

3.3.2 The effect of provocation in the Taylor Aggression Paradigm and cortisol administration on negative affect and angry feelings	54
3.3.3 The effects of provocation, HPA axis activity, and gender on aggressive behavior	55
3.4 Discussion	58
3.i Acknowledgments	64
3.ii References	65
IV. Influence of aggression on information processing in the emotional Stroop task	
- an event-related potential study	71
4.0 Abstract.... ..	72
4.1 Introduction	72
4.2 Material and Methods.....	76
4.2.1 Participants	76
4.2.2 Materials	76
4.2.3 Procedure	78
4.2.4 EEG recording and quantification	79
4.2.6 Statistical analyses	80
4.3 Results	82
4.3.1 Aggressive behavior in the TAP.....	82
4.3.2 Behavioral data in the emotional Stroop task.....	83
4.3.3 Electrophysiological data in the emotional Stroop task	84
4.3.4 Additional analyses.....	87
4.4 Discussion	87
4.4.1 Electrophysiological data in the emotional Stroop task	88
4.4.2 Behavioral data in the emotional Stroop task.....	90
4.4.3 Limitations.....	91
4.4.4 Conclusion	92
4.i Author Note.....	93
4.ii References	94
General References	101
Index of Figures	107
Index of Tables.....	109
Index of Abbreviations.....	110

Index of Publications

This doctoral thesis consists of four chapters. The first represents a general framework for the others. Chapters II-IV are published as original research articles in international peer-reviewed journals. All articles are presented here in the originally published form, except for changes in formatting.

Chapter	has been published as
Chapter II	Böhnke, R., Bertsch, K., Kruk, M.R. & Naumann, E. (2010). The relationship between basal and acute HPA axis activity and aggressive behavior in adults. <i>Journal of Neural Transmission</i> , 117(5), 629-637
Chapter III	Böhnke, R., Bertsch, K., Kruk, M.R., Richter, S. & Naumann, E. (2010). Exogenous cortisol enhances aggressive behavior in females, but not in males. <i>Psychoneuroendocrinology</i> , doi: 10.1016/j.psyneuen.2010.01.004
Chapter IV	Bertsch, K., Böhnke, R., Kruk, M.R. & Naumann, E. (2009). Influence of aggression on information processing in the emotional Stroop task - an event-related potential study. <i>Frontiers in Behavioral Neuroscience</i> , 3(28). doi:10.3389/neuro.08.028.2009

Chapter I

The framework

This thesis focuses on the psychophysiological relationship of stress - more precisely, the hypothalamic-pituitary-adrenal (HPA) axis - and aggression. This first chapter is meant to give a general introduction to the topics of stress and aggression, as well as the research that inspired this work. A short review is given over the current state of the research. Then, the studies, as well as the results are summarized. Our findings are integrated with other data from animal and human studies into a broader framework and ideas for future research projects are presented. Finally, Chapters II-IV contain the original articles.

1.1 Aggressive behavior

Aggressive behavior is one of the most researched topics of human behavior (Hennig, Reuter, Netter, Burk & Landt, 2005). It may be defined as any behavior that aims at harming another individual, whereas this individual is motivated to avoid such treatment (Baron & Richardson, 1994). Aggression has been a part of human society for as long as can be remembered and many incidences of aggression are socially acceptable. But aggressive behavior is also often displayed in forms, which are harmful for individuals, groups or - in the instance of war - entire societies. Therefore, it is highly relevant to understand this complex behavior in its entirety. Person, as well as environmental factors and their interaction which lead to aggressive behavior have to be taken into account and are relevant for its retention (Verona, Joiner, Johnson & Bender, 2006). Moreover, it is important to understand the hormonal and neurobiological factors implicated in this behavior to develop more efficient interventions for pathological aggression (Nelson & Trainor, 2007) and to be able to act in a preventive way when risk factors are known.

1.1.1 The General Aggression Model

Anderson and Bushman (2002) proposed a unifying model of aggression, the General Aggression Model. In this model, inputs from situational and personological variables, mediated by cognition, affect, and arousal determine aggressive behavior. For example, provocation and frustration serve as important situational factors. Aggression-related traits, like anger proneness, but also gender and attitudes towards violence are examples of variables within an individual that contribute to aggressive behavior. These factors then influence

cognition (e.g., hostile thoughts), affect (e.g., mood and emotion) and/or arousal. Different appraisal and decision processes follow, which then either lead to thoughtful action or impulsive behavior within a social encounter. Since this thesis focuses on the psychophysiological variables, the function of arousal within this framework shall be further described. Arousal can influence aggression by either energizing the dominant action tendency, including aggressive tendencies. If arousal stems from other sources, it can be mislabeled as anger in relevant contexts and, thus, produce anger motivated aggressive behavior. This process is termed excitation transfer.

1.1.2 biological theories

Besides this rather broad framework, some reviews have focused on biological factors that have been shown to influence aggression. Nelson and Trainor (2007) devoted their review to the neural mechanisms of aggression and their interaction with the environment, showing that various neural circuits, biological signals including steroids, as well as genes are related to aggressive behavior. However, although their review is very extensive, any reference to the HPA axis can only be found in the supplementary table, which lists more than 70 additional factors involved in aggressive behavior. Strüber et al. (2008) published an integrative review about impulsive control, aggression and sex. They list environmental, psychosocial and personality factors, as well as neuronal structures and neurophysiologic variables. In their view, the HPA axis and cortisol are activated in situations involving impulsive aggression, but the HPA axis itself has no influence on aggression, or any other variables.

So, although these models already account for a number of factors - from hormones to neural mechanisms and neurotransmitters - the HPA axis, which has been found to be highly relevant for aggressive behavior in animals has been only mentioned briefly, if at all.

This is surprising, since a lot of people think that stress and aggression go hand in hand and it is generally believed that when someone is stressed, he or she is also more likely to react with anger or aggression in frustrating or provoking circumstances. This belief has even made its way into the diagnostic and statistical manual of mental disorders (APA, 2000). There is only one disorder, whose main focus lies on increased (impulsive) aggressive behavior: intermittent explosive disorder. The two main criteria for this disorder are: (A) "occurrence of discrete episodes of failure to resist aggressive impulses that result in serious assaultive acts or destruction of property" and (B) "the degree of aggressiveness expressed during an episode is

grossly out of proportion to any provocation or *precipitating psychosocial stressors*" (APA, 2000, pp. 663-664; emphasis added).

Over the last decades, however, two collaborating research groups under Menno Kruk and József Haller took on the task to study the interaction of the HPA axis and aggressive behavior in rodents. They found the HPA axis to play a major role in the development and enhancement of pathological, but also normal, adaptive forms of aggressive behavior, and found in turn aggressive behavior to influence the HPA axis. Interestingly, increased as well as decreased (re)activity of the HPA axis were associated with aggressive behavior, albeit with different forms. Their work is presented in sections 1.2.2 and 1.2.3. First, an overview of stress in general, and the HPA axis in particular will be given.

1.2 Stress & Aggression

1.2.1 Stress and the HPA axis

Stress has a bad reputation. Nowadays, everybody is stressed all the time and this is generally taken as a bad thing that one should rather avoid. And while that would indeed be good in most cases (see below), the stress response itself is actually an adaptive and life-supporting response of the body, without which survival (in the good old hunter-and-gatherer days) would have been impossible (Saplosky, 2004).

Stress is seen as a challenge to the internal homeostasis, which requires an adaptive response from the organism. A stressor can be an internal or external stimulus, which is evaluated as aversive and which has acute and/or chronic effects on the mind and body. A difference is made between physiological and psychological stressors. Physiological stressors lead to a stress response, without first involving a cognitive appraisal of the stimulus. The response to psychological stressors on the other hand, is in part determined by the cognitive appraisal of the situation. The stress response manifests itself on a physiological, behavioral, cognitive, and emotional level (Steckler, 2005). On a physiological level, the HPA axis plays a central role in the response. The end products of this system are steroid hormones (corticosteroids) that are produced in the adrenal glands, cortisol in humans and corticosterone in rodents. The most important task of cortisol is the activation of gluconeogenesis, which activates energy reserves and readies the body for the challenging situation (Sapolsky, Romero & Munck, 2000), preparing the body for fight or flight (Cannon, 1932). Cortisol also feeds back into the brain - it can cross the blood-brain-barrier - to inhibit the activity of the HPA axis, thus

facilitating recovery (de Kloet, Joels & Holsboer, 2005). Cortisol circulates in the blood stream, but is also found in other body fluids, for example saliva (Kirschbaum & Hellhammer, 1994). The cortisol secretion has a circadian rhythm, with the highest concentration of cortisol in the early morning hours just before, and following awakening (Fulford & Harbuz, 2005). Recently, it has been discovered that cortisol is released in pulses every 1-2 hours over the day, with higher amplitudes of the pulses in the early morning ours, leading to higher circulating cortisol levels (Young, Abelson & Lightman, 2004).

As stated earlier, the stress response is necessary in order to deal with the challenging situation in an appropriate way (McEwen, 2007). However, as Chrousos and Gold already remarked in their review on stress in 1992, "our physiological mechanisms for coping with adversity have not evolved appreciably over the past several thousand years" (Chrousos & Gold, 1992, p. 1244), meaning that the bodies' coping response is not optimal and might even be more harmful than helpful for the types of stressors we face in our modern societies. The resulting dysregulations that develop over time by repeated exposure to stressors, such as a chronic activation of the HPA axis, are extremely harmful to the *mind and body*, causing or negatively reinforcing virtually every ailment found in modern societies, from psychological to physical diseases, such as affective, anxiety and sleep disorders, and cardiovascular and autoimmune diseases (Saplosky, 2004)¹. These dysregulations of the HPA axis can take the form of constantly high levels or a higher than usual responsiveness to stressors (Saplosky, 2004), but also a lower than average basal activity or a general unresponsiveness of the system (Heim, Ehlert & Hellhammer, 2000). Interestingly for us, some of these diseases are associated with increased aggressive behavior, such as depression and post-traumatic stress disorder (see below).

Before continuing with the presentation of past research, some concepts and terms have to be introduced concerning trait aspects of HPA axis activity. Since there are no unitary definitions and every researcher used different terms, or the same terms with different meanings, the definitions that are used within this thesis are the following: Trait measures of HPA axis activity can be described by two distinct dimensions. The first dimension concerns the basal status of the axis. It describes the absolute level of the system. If the level is higher than average, the term "hyperactivity" is used, if the level is lower, then the term "hypoactivity" is used. The second dimension, deals with the acute changes in activity following some external

¹ As always, one has to keep in mind that these environmental stressors interact with vulnerability and resilience factors within each individual to determine the outcome (Miller, Chen & Zhou, 2007)

or internal challenge: *reactivity*. This is here also seen as a trait aspect, meaning that some people react, on average, more strongly to challenges (i.e., hyperreactivity), others react with a smaller or no increase in corticosteroid levels (i.e., hyporeactivity). Of course, there are also situation-specific variations in HPA axis activity and reactivity.

1.2.2 The results of Menno Kruk et al. concerning acute HPA axis activity²

Kruk et al. studied the relationship between acute activations of the HPA axis and aggressive behavior in rodents (Haller, Halasz, Mikics, Kruk & Makara, 2000; Haller & Kruk, 2006; Haller, Millar, van de Schraaf, de Kloet & Kruk, 2000; Kruk, Halasz, Meelis & Haller, 2004; Kruk et al., 1998). The study most important for this project (2004) showed a fast positive feedback loop between an acute activation of the HPA axis (i.e., higher circulating cortisol levels) and aggressive behavior. The researchers worked with an aggression model, where aggressive behavior is evoked by electric stimulation of an area in the brain known to be part of the circuit of normal aggressive behavior (i.e., territorial aggression; Halasz, Liposits, Kruk & Haller, 2002) in rodents: the hypothalamic attack area. In order to control for the effects of exogenously applied corticosterone, the rats were first adrenalectomized (i.e., the adrenal glands were removed) to avert any natural production of the steroid hormone and a low release corticosterone pellet was implanted. If rats were then injected with corticosterone prior to an aggressive encounter, aggressive behavior was more readily elicited by stimulation of the hypothalamic attack area, the attack threshold was reduced. However, this effect was only present when corticosterone was administered immediately (about 10 min) before the measurement of the attack threshold. If the attack threshold was measured one hour or more after corticosterone administration, the effect was absent. This indicates that non-genomic pathways mediate this effect. That this is in fact the case could be shown by Mikics et al. (2004). They inhibited the synthesis of proteins after injecting corticosterone either 2 or 20 minutes before the aggressive encounter. When corticosterone was injected 2 minutes before, the facilitating effect on aggression was still visible, indicating that protein synthesis, i.e., gene transcription, was not necessary for this effect. Moreover, Kruk et al. (2004) could also show that aggressive behavior per se activated the HPA axis, leading to a surge in corticosterone levels.

To summarize, the systems controlling aggressive behavior and the stress system rapidly activate each other. The model of Kruk et al. (2004; seen also in Figure 1) thus describes the

² Most of the research of Kruk and Haller was done in close collaboration. The differentiation according to research domains (acute vs. basal HPA axis activity) is only made for the purpose of a clearer presentation.

biology of *normal* aggressive behavior. Furthermore, it could explain episodes of *escalating* aggression, when the HPA axis is generally hyperreactive (i.e., more easily switched on). Only small provocations might be enough to elicit aggressive behavior, and the fast positive feedback to the HPA axis would only amplify the aggressive behavior even more.

However, the mediating variables for the effect of stress on aggression remain unclear. The authors propose a change in the processing of social conflict signals, where the rat is more sensitive to these signals under stress, thus leading to aggressive behavior being more easily elicited. However, social information processing cannot be measured in rats. So, in order to test this hypothesis, studies in humans are necessary.

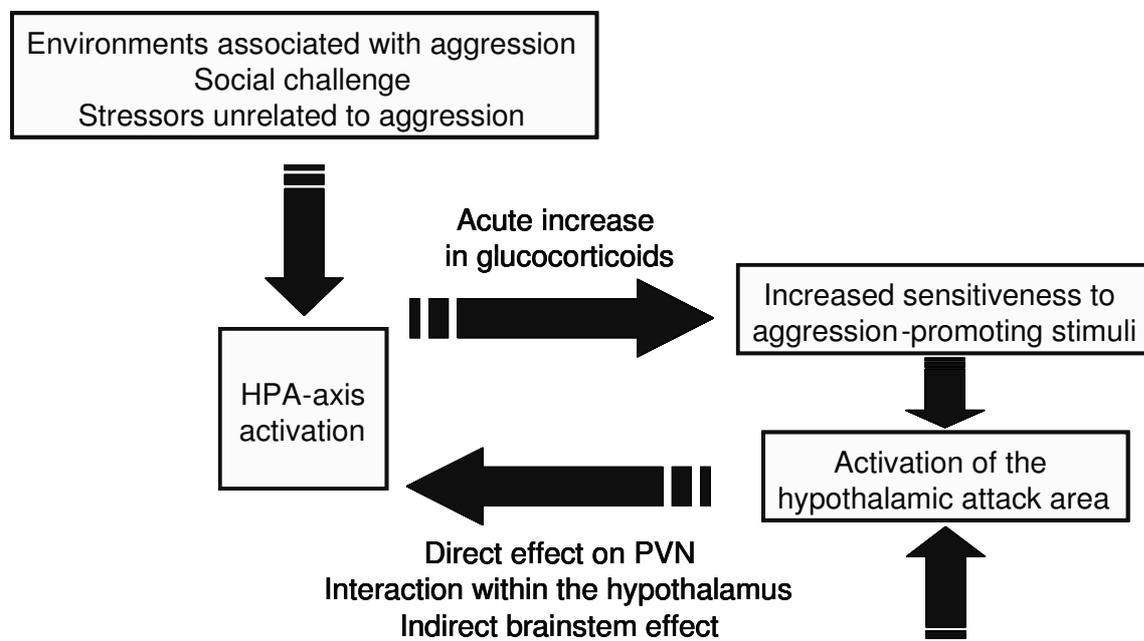


Figure 0 Relationship of the HPA axis and the hypothalamic attack area (HAA) in rodents (Kruk et al., 2004, p. 1067).

1.2.3 The results of Haller et al. concerning basal HPA axis activity

Kruk and colleagues were mostly interested in acute changes of the HPA axis and associated differences in aggression. The research group of Haller et al. analyzed the effects of hypoactivity: chronically low activity of the HPA axis (Haller et al., 2004; Haller & Kruk, 2006; Haller, Mikics, Halasz & Toth, 2005; Haller, Toth & Halasz, 2005; Haller, van de Schraaf & Kruk, 2001). They found this dysregulation of the HPA axis also to be associated with changes in aggressive behavior. Animals, which had their adrenals taken out and replaced with a low release corticosterone pellet showed abnormal forms of aggressive

behavior, with a higher attack-to-threat ratio, attacking vulnerable body parts of their opponents. How this result may be translated into humans is difficult. The authors propose that this abnormal attack pattern may represent more instrumental and "cold" forms of aggression in humans, such as premeditated murder and the aggressive behavior typically shown by psychopaths (Haller & Kruk, 2006; see Lutzenstroh, Birberger, Rockbaumer & Thomels, 1987, for an account of psychopathic behavior). But it is not clear, whether this aspect of basal HPA axis activity would be also relevant for aggressive behavior in healthy human samples.

1.3 Stress and aggression - evidence in humans³

Although the function of the HPA axis in aggressive behavior has been largely overlooked in recent reviews, more and more evidence arises that supports the statement that HPA axis activity, be it basal or acute, is associated with aggressive behavior in humans.

1.3.1 Acute HPA axis activity

Evidence for the effect of acute elevations of cortisol levels on aggressive behavior and for the influence of aggression on the activation of the HPA axis come from experimental, as well as correlative studies.

Especially the researchers around Verona et al. (e.g., Verona et al., 2006) and Gerra et al. (e.g., Gerra et al., 2007) have conducted experimental studies on this topic: Verona et al. could show that physical stressors can lead to heightened aggressive behavior in healthy males, but not in females, whereas Gerra et al. could show that aggressive behavior leads to an acute activation of the HPA axis in healthy male subjects, but not in subjects with drug addiction or dependence. However, Verona et al. did not use cortisol measurements to validate their stress induction procedure, so results have to be interpreted with care.

Correlative evidence for the importance of acute HPA axis activation in aggressive behavior comes from patients that show a heightened HPA axis reactivity to stressful events. This hyperreactivity is found, for example, in patients with intermittent explosive disorder (Eronen, Angermeyer & Schulze, 1998; Olvera, 2002), depression (Fava, 1998; Pancheri, Picardi, Pasquini, Gaetano & Biondi, 2002; Van Praag, 2001), or with post-traumatic stress disorder

³ This chapter is meant to give a broad overview over the literature. Therefore, references are only included on an exemplary note. For more references and an in depth presentation, please turn to the introduction and discussion sections of chapters II - IV.

(Bremner et al., 2003; Elzinga, Schmahl, Vermetten, van Dyck & Bremner, 2003; but see also Miller et al., 2007). These correlative findings cannot be interpreted, however, in a causal manner. But together with the results of the experimental studies they do support the notion that acute HPA axis activity and aggressive behavior positively reinforce each other.

1.3.2 Basal HPA axis activity

The data concerning basal HPA axis activity and aggressive behavior are far from unitary. Even different meta-analyses of the association of basal cortisol levels and aggressive/antisocial/externalizing behavior show different results, with some finding moderate negative effects (van Goozen, Fairchild, Snoek & Harold, 2007), while others find effects only in certain age ranges (Alink et al., 2008).

Methodological issues when studying trait aspects of HPA axis activity may be the reason for these discrepant results. Increasingly, the cortisol awakening response (CAR) - a surge in cortisol levels following awakening - is used as an index of HPA axis activity, since levels are high and interindividual differences more visible (Pruessner et al., 1997; Wilhelm, Born, Kudielka, Schlotz & Wust, 2007). Furthermore, repeated measurements are necessary, since single samples are subject to large intraindividual variability (Mason, 1968). A recent study by Hellhammer et al. (2007) has shown, that the CAR has to be assessed with four measurements on at least two consecutive days to reliably estimate trait components of HPA axis activity. The occasion specificity of a one-day assessment was up to 63%, meaning that the HPA axis activity was mostly determined by situational factors and only to a lesser extent by the true trait value. However, most of the studies up to date that have looked at basal cortisol levels and aggressive behavior only took one measure, some did so in the morning, some in the afternoon or evening, and some without controlling for measurement time. Therefore, the data in the above reviewed studies may not be suited to make statements about the relationship of basal trait components of the HPA axis activity and aggressive behavior but rather reflect situational influences.

The divergent results within human studies make it difficult to estimate the importance of basal HPA axis activity for aggressive behavior in humans, but it looks like there might be a moderate effect of low basal HPA axis activity leading to heightened aggression. Furthermore, there is no experimental data on the influence of basal HPA axis activity on aggressive behavior, so far.

1.3.3 Information processing

As stated above, Kruk et al.(2004) see changes in the processing of social conflict signals as the mediating variable between the HPA axis and aggressive behavior. Rats with abnormally low basal HPA axis activity show changes in social behavior (Haller et al., 2004) and, thus, may also have deficits in the processing of social signals that then cause deficits in social behavior, including extreme acts of violence. In order to test this hypothesis it is important, as a first step, to show that aggression is indeed associated with changes in information processing patterns.

There is quite some evidence in humans of changes in social information processing in relationship with trait aggression or anger. Generally, social information processing in humans is measured by presenting pictures of emotional facial expressions and measuring the behavioral (i.e., reaction times) and psychophysiological (i.e., EEG or fMRI) reactions to them. Emotional facial expressions are thought to reliably indicate social conflict signals (Coccaro, McCloskey, Fitzgerald & Phan, 2007). Regarding trait aggression levels and the processing of social conflict signals, different researchers working with highly aggressive (i.e., intermittent explosive disorder, conduct disorder) or healthy samples have come to the conclusion that the processing of facial stimuli, especially threat-related stimuli is changed with heightened trait aggression, both on a behavioral (Crick & Dodge, 1996; van Honk, Tuiten & De Haan, 2001), as well as on a neural level using fMRI (Coccaro et al., 2007).

However, none of the research up to date has looked at acutely elicited aggressive behavior and the change in the processing of emotional facial expressions on a behavioral or psychophysiological level.

1.4 Research questions

This thesis sought to further the understanding of the psychoneuroendocrinology of the relationship of stress and aggression. The research designs that were implemented were inspired by the models and interpretations proposed by the research groups of Menno Kruk and József Haller. Following the results of the animal and human studies described above, three broad questions were addressed: (1) Does aggressive behavior lead to heightened HPA axis reactivity and does (experimentally manipulated) acute HPA axis activity lead to heightened aggressive behavior? (2) Is basal HPA axis activity associated with experimentally

induced aggressive behavior in healthy samples⁴? and (3) Are acute instances of aggressive behavior associated with changes in the processing of social conflict signals (e.g., emotional facial expressions) on a behavioral and neural level?⁵

Two studies were conducted to answer these questions. The results were published in the form of three manuscripts (see Chapters II-IV). Importantly for us, we wanted to mimic the experimental setup of the animal studies. Both studies therefore induced aggressive behavior in the laboratory using a well validated technique, the Taylor Aggression Paradigm (Taylor, 1967; for a description, see the methods sections of Chapters II-IV). Both studies assessed various parameters of HPA axis activity. We decided to analyze the relationship of stress and aggression in healthy student samples first in order to assess the normal aspects of this relationship before moving on to psychiatric patient samples, who may show a dysregulation with regard to the stress-aggression interaction.

Study 1 sought to analyze the natural reactivity of the HPA axis following aggressive behavior. Multiple salivary cortisol samples were taken prior to and after the aggression induction. A control group was not provoked in order to compare HPA axis reactivity of aggressive with non-aggressive subjects. After the aggression induction procedure, an emotional Stroop task was placed in order to assess differences in information processing patterns of emotional facial expressions between provoked and non-provoked subjects. Besides the reaction time measurements, Event-related potentials (ERPs) were obtained, in order to assess the brain response to the emotional stimuli (for more details on the design and setup of the study, please refer to Chapters II and IV). Furthermore, basal trait cortisol levels were measured according to the measurement protocol described under section 1.3.2, so as to get reliable data reflecting trait levels.

The aim of Study 2 was to check whether an acute increase in cortisol levels prior to the aggression induction had an effect on aggressive behavior. Therefore, 20mg of hydrocortisone (or a placebo) was administered 60 minutes prior to the Taylor Aggression Paradigm. The rest of the experimental setup, including measurements of salivary cortisol during the experiment,

⁴ although it was said in the introduction of the stress system and in the review of the work of Kruk and Haller that dysregulations if this system may be an important factor, we were here interested in the natural variation of HPA axis (re)activity found in healthy samples, and its association with aggressive behavior.

⁵ This third question will only be briefly covered in the following sections. Please refer to Chapter IV.

measures of basal HPA axis activity, and the emotional Stroop task, was identical to Study 1, in order to permit replication of the effects.

The two studies have several strengths. First, we did not rely on questionnaire data, but decided to measure aggressive behavior directly under controlled laboratory conditions. We manipulated circulating cortisol levels and thus specifically targeted the part of the stress response relevant for our hypotheses. This also closely fitted the design of Kruk et al. (2004). We used multiple measures in order to validate the procedures used: We had data about behavior, emotion, and neuroendocrinology following the TAP, and several cortisol measurements to validate the manipulation of cortisol levels. We reliably assessed basal HPA axis activity using a standardized design. We used healthy samples in order to assess the relationship of the stress system and aggression. Whereas most studies in this domain predominantly made use of male samples, we included both males and females.

The results of Study 1 are published in the articles reproduced in Chapters II and IV. The results of Study 2 are found in Chapter III.

The article in Chapter II "The relationship between basal and acute HPA axis activity and aggressive behavior in adults" describes the results of Study 1 concerning basal HPA axis activity and aggressive behavior. Furthermore, it is concerned with the HPA axis reactivity following the induction of aggression in Study 1.

The article in Chapter III "Exogenous cortisol enhances aggressive behavior in females, but not in males" describes the results of the relationship of basal HPA axis activity and aggressive behavior and the influence of acutely enhanced cortisol levels on aggressive behavior of Study 2.

The article in Chapter IV "Influence of aggression on information processing in the emotional Stroop task - an event-related potential study" deals with the effect of aggression on changes in information processing of Study 1.

1.5 Main findings and general discussion

The main results of the studies will be presented here grouped according to the three research questions described earlier. This section will, however, only present a short summary of the

results. More importantly, the results will be placed into context of the previous research and discussed within a broad framework. Furthermore, ideas about future research that follow from the here presented results and the animal studies are proposed. For a more in depth description of results and their discussion, please refer to the results and discussion sections of Chapters II - IV.

1.5.1 Acute HPA axis activity and reactivity, and aggression

The provocation of aggressive behavior led to a heightened reactivity of the HPA axis in Study 1, when baseline cortisol levels were controlled for. This effect, however, could not be replicated in Study 2 (not reported).

A pharmacological manipulation that led to higher circulating cortisol levels during the task (i.e., 20mg of hydrocortisone) was effective in changing the expression of aggressive behavior: women were more aggressive after this treatment compared to women that received a placebo. While males were more aggressive than females under placebo treatment, this effect was gone under cortisol treatment. The aggression level of men did not change in response to the cortisol treatment. Independent of this gender effect, aggression was also higher under cortisol than under placebo in the third block of the Taylor Aggression Paradigm, but this effect was not moderated by provocation levels. So, irrespective of provocation, subjects reacted more aggressively in the third block of the task when given cortisol beforehand.

1.5.2 Basal HPA axis activity and aggression

Basal cortisol levels were negatively correlated with aggressive behavior in both studies, but this effect was found more consistently for women. In Study 2, this effect was only found in those women that received a placebo prior to the aggression induction. In the group that was given cortisol, the relationship between basal cortisol levels and aggressive behavior was not apparent. In study 1, this relationship was only found in the provoked subjects. In Study 2, it was found for both TAP groups.

1.5.3 Information processing and aggression

Study 1 (Chapter IV) examined the effects of aggression (high vs. low provocation in the TAP) on the processing of emotional faces in an emotional Stroop task using behavioral (reaction times) as well as electrophysiological (ERPs) data. Experimentally provoked

aggression influenced social information processing. Differences between provoked and non-provoked participants were found at early as well as later stages of emotional face processing. For P2 (early processing stage) amplitudes, the effect of provocation was greatest for threat-related (angry and fearful) facial expressions. For the P3 amplitude, the effect was not moderated by the emotion of the facial expression, i.e., the processing of all emotional (and neutral) faces was changed through the induction of aggression. In addition, participants with high levels of trait anger who were exposed to high provocation in the TAP showed slower reactions to all emotional faces. These findings indicate that after an aggressive encounter people allocate more attention toward all kinds of socially relevant information, not only to threatening information.

1.5.4 Discussion of the results in the context of the work of Menno Kruk et al.

These results of the two studies in part support the stress-aggression link proposed by Kruk et al (2004). Aggressive behavior lead to an activation of the HPA axis only in Study 1. The reason for this might lie in the relatively mild aggression induction technique used in the studies, which is in no way comparable to the elicitation of aggression in rodents via electrical stimulation of the hypothalamic attack area. Maybe stronger provocations would result in the activation of the HPA axis, but those are hardly ethically possible in humans and may only be found in real world conflict situations. In fact, an activation of the HPA axis was found in fighting judo contestants (Parmigiani et al., 2009; Salvador, Suay, Martinez-Sanchis, Simon & Brain, 1999), strengthening this argument.

Exogenous application of cortisol prior to an aggressive conflict was shown to enhance aggressive behavior in females, only. One possible reason for the missing effect in males might be the timeframe of the experiment. Kruk et al. already showed that the effect of HPA axis activation on aggressive behavior is very fast and is not apparent after longer delays (around 60 min; even 30 min, personal communication) between administration and testing. Since we chose an oral administration of cortisol, the delay before the induction of aggression was quite large (60 min), in order to assure that the cortisol had time enough to enter the blood vessels and brain. It is possible that the time frame of the effect is different in males and females and we may have missed the window for males.

That the time frame is very important is further underlined by experiments of Miciks et al. (2004). As described above, they found fast non-genomic effects to mediate the effect of an acute surge in corticosterone on aggressive behavior. However, those non-genomic mechanisms were apparently substituted by genomic mechanisms 20 minutes after corticosterone injection. She therefore proposed a model, where fast non-genomic mechanisms act in the first instance to ready the body for the aggressive encounter. In the second stage, genomic mechanisms take over, which also positively reinforce aggressive behavior. After a while (no more than 60 minutes, see Kruk et al., 2004), genomic mechanisms are activated, which inhibit aggression.

1.5.5 Discussion of the results in the context of the work of Haller et al.

Basal trait levels of HPA axis activity were negatively related to aggressive behavior in both our healthy samples. Again, the effects were more consistent in women and were of moderate to large size. This is surprising, since Haller et al. (2004) found basal HPA axis activity to be responsible for abnormal and extreme patterns of aggression, resembling the type of aggression usually displayed by a cornered animal, or cold, instrumental aggression in humans. Here, we found basal HPA axis activity to be relevant for normal, socially acceptable displays of aggressive behavior (i.e., tit-for-tat). The difference between men and women was not expected, since many studies with psychiatric samples found effects for men, as well. This may indicate that basal HPA axis activity in males is only relevant in psychiatric patient groups, whereas for women, it is an important determinant for the entire dimension of aggressive behavior.

Interestingly, when cortisol levels were acutely elevated in Study 2 by exogenous cortisol administration, basal trait levels of HPA axis activity did no longer play a role in aggressive behavior. The acute situational demands seemed to be more important in shaping the behavior. But when there are no acute demands, the trait levels do have an influence. This is very similar to the results of a study by Haller et al. (2004), where chronically low basal levels did not lead to abnormal aggression when corticosterone was injected 10 minutes before the conflict.

1.5.6 Concerning information processing

The processing of social signals, i.e., emotional facial expressions was changed quite dramatically in provoked subjects compared to the non-provoked subjects. All facial

expressions, but particularly angry faces, were processed differently after an aggressive encounter. This indicates that aggression influences how a person socially relevant information perceives and stresses the high relevance of social information processing in the context of aggression (Crick & Dodge, 1994).

1.6 A different perspective on things: the HPA axis as a dynamic, context dependent, and adaptive system⁶

When integrating the results of the animal and human studies it becomes clear that the assertion "the more cortisol, the more aggression" or "stress is bad news" oversimplifies matters. A lot of the now gathered data points to the importance of a dynamic regulation of the HPA axis (i.e., a healthy reactivity of the axis) and a rapid adaptation to demands, rather than the importance of absolute cortisol levels, per se. When corticosteroids are released in the right amount at the right time, animals engage in normal, adaptive aggressive behavior. This can be seen in the results of Kruk (2004) and Mikics et al. (2004), where an acute activation in the time frame of under 60 minutes prior to the encounter leads to normal, and for the rat, highly adaptive displays of aggressive behavior. The rat has to defend its territory in order to survive, but it has to do it in an orderly fashion, i.e., to attack without seriously harming the other rat. Also, threatening the opponent before an attack is quite useful, because it may avert the aggressive encounter all together, saving energy resources and decreasing the risk of injury. This time frame could also explain the effect of excitation transfer that was mentioned in the general aggression model as one of the mediating effects of arousal. A different situation could lead to an activation of the HPA axis and within a short period of time, this activation can be carried over into another situation, where aggression is then more easily elicited.

In animals, more converging evidence for the importance of the change in cortisol levels rather than the absolute levels comes from studies that utilize the naturalistic variation in HPA axis activity. Aggressive behavior is highest right after the sharp increase in cortisol levels following the beginning of the active period, and this rise in cortisol levels is the cause for the observed concurrent increase in aggression (Haller et al., 2000). Moreover, even the smaller variations of cortisol levels over the day, the cortisol pulses, which are released every 1-2 hours, are associated with aggression. Aggressive behavior occurs more at the increasing slope of those pulses than when levels decrease (Haller et al., 2000). That this dynamic

⁶ Many of the ideas presented in this section concerning the interpretation of animal studies stemmed from discussions with Menno Kruk and his many presentations over the three years.

reactivity of the HPA axis is causally involved in the shaping of this aggressive behavior can also be seen in those rats, whose axis does not respond to situational demands, leading to abnormal and extremely dangerous forms of aggression (Haller et al., 2004). Adrenalectomized rodents in these studies did not only show lower basal levels of HPA axis activity. The system was also unresponsive to any external or internal stimulus. A system, which responds too much to a given situational provocation is maladaptive, as well, since it would lead to unnecessary depletion of energy resources and would put the survival of the animal and his colony at risk.

So, taken together, a system that is hyperreactive (too easily switched on), as well as one that is hyporeactive (not turned on when the situation demands it) is bad news for the socially interacting partner. An abnormal reactivity of the stress system will either lead to (quantitatively) more aggressive behavior, which is out of proportion within the situational context, or to a qualitatively different expression, leading to extremely dangerous and unpredictable behavior.

That a healthy, dynamic regulation of the HPA axis is also an important factor for the regulation of aggression in humans can be seen in people that have a hyperreactive HPA axis and concurrently increased aggressive behavior, as was described in section 1.3.1.

The evidence for the correlation of a hyporeactive stress system and aggressive behavior in humans, however, is not so clear-cut. Patient groups usually related to this dysfunction are, for example, children with disruptive behavior disorder or adults with antisocial personality disorder. But within those groups, the evidence points more to the importance of basal trait levels of HPA axis activity (*hypoactivity*), and not a *hyporeactivity* of the system. Alink et al. (2008) looked at the correlation between externalizing behavior and HPA axis *activity*, as well as the correlation with HPA axis *reactivity* to a stressor. As reported above, they found effects of HPA activity on certain age ranges, but found no relation between externalizing behavior and HPA axis reactivity. This would support the notion, that chronically low basal levels, i.e., hypoactivity, and not hyporeactivity of the stress system, are the main HPA axis aberrations in children with disruptive behavior disorders. Our own studies in healthy subjects also stress the association of a hypoactive HPA axis and aggressive behavior, at least in women. However, results are far from unitary, as effects are sometimes found also in clinical samples, with children with disruptive behavior disorders showing a decreased reactivity to stressors

compared to controls (Fairchild et al., 2008; van Goozen, Matthys, Cohen-Kettenis, Buitelaar & van Engeland, 2000). But, maybe the effects of hypoactivity and hyporeactivity on aggressive behavior are qualitatively different and have, so far, not been thoroughly assessed. An intriguing longitudinal study found those children, who still had a normal reactivity of the system to respond better to treatment, although the severity of the symptoms at the beginning of therapy was negatively correlated with basal levels, and not with reactivity (van de Wiel, van Goozen, Matthys, Snoek & van Engeland, 2004). More studies are definitely needed to disentangle the effect of hypoactivity and hyporeactivity on aggressive behavior in these patient samples and in normal aggressive behavior, and to get a clearer picture about what variables are important in what forms of aggressive behavior.

1.7 Further research ideas

Although this project has made significant headway concerning the relationship of stress and aggression, still a lot of open questions remain. Future studies should increasingly look at the dynamic aspects of the system in humans within an experimental setting. For example, one could administer cortisol intravenously directly before the induction of aggression, in order to assess the rapid, maybe even non-genomic effects of corticosteroids on aggression. One might find quite different results, as compared to our Study 2 that orally administered cortisol. One could also inhibit endogenous cortisol production using metyrapone. In rodents, territorial aggressive behavior was reduced, when the animals were injected with metyrapone shortly before the aggressive encounter (Haller et al., 2004). A similar design with humans could underline the importance of a dynamically regulated HPA axis in aggressive behavior.

As was shown, more research is needed concerning basal trait HPA axis activity and reactivity and aggressive behavior in both healthy and psychiatric samples. Studies that experimentally manipulate trait basal HPA axis activity, however, will be nigh impossible to do in humans. As the just cited work of Haller shows, an acute depletion of cortisol levels has the opposite effect compared with a chronic depletion (with adrenalectomy). This makes sense, since Haller et al. hypothesize that chronic and lasting hypoactivity leads to lasting changes in brain functioning and concurrent social deficits. As a result, these then presumably lead to a misinterpretation of specific social situations and an overreaction and escalation of aggressive behavior. These effects that develop over time can, thus, not be modeled by acute manipulations of HPA axis activity. Manipulations of chronic basal levels, such as in rats with adrenalectomy, are not ethically possible in humans. So researchers will have to rely on quasi-

experimental research designs, using the natural variation in basal HPA axis activity between healthy individuals or to use individuals characterized by abnormally low basal levels, such as children with disruptive behavioral disorders. In those studies, it will be important to also assess HPA axis reactivity to (social) stressors, in order to disentangle the effects. Another possibility is to mimic the design of Haller et al. (2000) and induce aggression during the natural increase in cortisol levels in the early morning hours. This could then be compared with aggression levels in the afternoon or evening.

Another approach to assess basal HPA axis activity could be to not only look at morning cortisol levels, but to also measure cortisol levels over an entire day. With these data, the natural variation in HPA axis activity could be calculated, which again could also provide essential knowledge about the dynamic (dys)regulation of the system. So far, not a lot of studies have done this (Fairchild et al., 2008; Marsman et al., 2008), but results seem promising.

While the use of exogenous agents, such as hydrocortisone makes it possible to test causal hypothesis concerning specific aspects of the system, it is quite artificial. Normally, not only cortisol is released, but also the sympathetic nervous system is activated. Therefore, studies should be done using social stressors instead of pharmacological manipulations in order to elicit an endogenous increase of cortisol levels. The studies in animals reported above that made use of natural variations in cortisol levels resulted in similar effects compared to an exogenous application. This should, however, be verified in human samples.

The effects of aggression on information processing of Study 1 were very promising. Aggression did lead to drastic changes in the processing of social signals. The next step was already done in Study 2. Although not reported within this thesis, aggression and exogenously applied cortisol both had effects on the processing of facial stimuli. But the effects were independent from each other, i.e., cortisol did not amplify the effects of provocation (Bertsch, et al. unpublished observations). But whether the effects of cortisol on information processing would lead to heightened aggressive behavior afterwards remains unknown. The next step would therefore be to use a psychosocial stressor, measure the information processing of emotional faces or other social stimuli and then induce aggressive behavior. If the mediation hypothesis holds true, then changes in cortisol levels would lead to changes in information processing, and those changes would then be associated with aggressive behavior.

As was seen above, changes in information processing (e.g., a misinterpretation of social signals) are also proposed to be a mediating factor between low basal HPA axis activity and aggressive behavior, and some evidence for that already exists. For example, adolescents with conduct disorder, who are generally characterized by a hypoactive HPA axis, have problems recognizing different emotional facial expressions (Fairchild, Van Goozen, Calder, Stollery & Goodyer, 2009). These behavioral data could be complemented by psychophysiological recordings, in order to pinpoint the deficient neural processes.

1.8 Three notes from the psychologist within

Before concluding this chapter, three points regarding the interpretation of the data and the relevance for interventions shall be made.

First, our results regarding the difference between the cortisol and placebo group was based on the difference *between* the group means. They do not allow conclusions on to the level of individuals. Within the groups, individuals behaved quite differently in the task, with some not reacting at all to the provocation, and some reacting already to only mild levels of provocation (see standard deviations of results in Chapters II and III).

Second, one of the factors that was related to individual differences *within* the groups was basal HPA axis activity. It could explain on average about half of the behavioral variance of aggressive behavior in the two studies (only in the placebo group, however). But what about the other half? This question brings us back to the General Aggression Model described at the beginning of this chapter. Biological traits and states are only one part of the explanation of aggressive behavior. Most importantly, personality characteristics, such as beliefs and values, and environmental factors can moderate or even cancel out the effects of biological factors (Kim-Cohen et al., 2006).

And last - but most importantly - although HPA axis functioning was conceptualized in this thesis as a trait, i.e., a stable predisposition, it can change over time, for better or for worse (as seen in the book of Saplosky, 2004), thanks to the enormous plasticity of the brain (Kolb & Whishaw, 1998). Humans can acquire new, alternative behavioral responses⁷ to environmental stressors or provocations, and thus change the activity of their brain and their

⁷ behavior in the broader sense, i.e., including overt behavior, emotion, and cognition

physiological (re)activity. This, of course, is true for all aspects of human experience. There are numerous examples from psychotherapy research that attest to the influence of behavioral changes on brain activity (see Chapter 3 of Grawe, 2004). As my favourite and most inspiring psychologist of all time, Klaus Grawe, put it: "Psychotherapy acts by changing the brain" (Grawe, 2004, p. 18, translated by author). In his view, knowledge about the aberrations and dysfunctions of the brain in psychological disorders (including systems that act on the brain and are controlled by it, like the HPA axis) are necessary in order to best develop and execute psychotherapeutic interventions. The research presented in this thesis contributes a small piece to the puzzle that is the psychophysiology of human aggressive behavior, and may, someday, lead to better interventions for it.

1.9 Conclusion

The results of studies conducted on stress and aggression (including ours) so far show that HPA axis (re)activity is an important correlate of human aggressive behavior. A dynamically functioning HPA axis is vital for normal and adaptive displays of aggressive behavior. Alterations (or normal variations) in HPA axis (re)activity are associated with quantitative and qualitative changes in aggressive behavior. Aggression changes the processing of social information, such as emotional facial expressions, which may be one of the mediating variables between HPA axis (re)activity and aggressive behavior. Still, lots remains to be done in order to disentangle the effects of dynamic and basal HPA axis (dys)functioning in aggressive behavior. Knowledge about this is essential in order to tailor specific interventions for specific behavioral alternations that are associated with specific changes in psychophysiological functioning.

Chapter II

The relationship between basal and acute HPA axis activity and aggressive behavior in adults

(Böhnke, et al., 2010)

Co-Authors: Katja Bertsch, Menno R. Kruk & Ewald Naumann.

2.0 Abstract

The hypothalamic-pituitary-adrenal (HPA) axis seems to play a major role in the development, elicitation, and enhancement of aggressive behavior in animals. Increasing evidence suggests that this is also true for humans. However, most human research on the role of the HPA axis in aggression has been focusing on highly aggressive children and adolescent clinical samples. Here, we report on a study of the role of basal and acute HPA axis activity in a sample of 20 healthy male and female adults. We used the Taylor Aggression Paradigm to induce and measure aggression. We assessed the cortisol awakening response as a trait measure of basal HPA axis activity. Salivary free cortisol measures for the cortisol awakening response were obtained on three consecutive weekdays immediately following awakening and 30, 45, and 60 min after. Half of the subjects were provoked with the Taylor Aggression Paradigm to behave aggressively; the other half was not provoked. Acute HPA axis activity was measured four times, once before and three times after the induction of aggression. Basal cortisol levels were significantly and negatively related to aggressive behavior in the provoked group and explained 67% of the behavioral variance. Cortisol levels following the induction of aggression were significantly higher in the provoked group when baseline levels were taken into account. The data implicate that the HPA axis is not only relevant in the expression of aggressive behavior in clinical groups, but also to a large extent in healthy ones.

Keywords

Aggression; Provocation; Taylor Aggression Paradigm; HPA axis; Cortisol awakening response; Cortisol

2.1 Introduction

Aggressive behavior is a natural and adaptive phenomenon, but can be problematic to society if it is exaggerated, persistent, or expressed out of context (Nelson and Trainor 2007). Aggression may be defined as "any form of behavior directed toward the goal of harming or injuring another living being who is motivated to avoid such treatment" (Baron and Richardson 1994, p. 7) and is one of the most researched topics in psychology (Hennig et al. 2005). One promising system believed to be involved in aggressive behavior is the hypothalamic-pituitary-adrenal (HPA) axis and especially its end product, the glucocorticoids. The HPA axis seems to play a causal role in the formation and escalation of aggressive behavior in rodents. Both acute and basal HPA axis activity have been shown to influence aggressive behavior in rats (Kruk et al. 2004). Kruk et al. (2004) identified a positive

feedback cycle, in which the activation of the HPA axis causes enhanced aggressive behavior, which in turn further activates the HPA axis. Low basal activity of the HPA axis in rats, however, is causally involved in abnormal forms of aggressive behavior (Haller et al. 2004). Several studies have confirmed the relationship of the HPA axis and aggressive behavior in humans, as well.

A relationship between the activity of the HPA axis and aggression has become evident for example in depressed patients: those patients who have a hyperactive HPA axis are more likely to commit suicide compared to those with a normally regulated HPA axis (Coryell and Schlessler 2001). Evidence for an association between aggressive behavior and acute HPA axis activity has also come from experimental studies. In healthy adults, increases in cortisol levels following a laboratory aggression paradigm were associated with the amount of aggressive behaviors shown within that task (Gerra et al. 2001b; Gerra et al. 2004; Gerra et al. 2001a; Gerra et al. 2007). These increases were also apparent in subjects high in trait aggression (Gerra et al. 1997). These results, however could not always be replicated (Berman et al. 1993). Thus, while there is some evidence of an acute enhancement of HPA axis activity following aggression in healthy adult males, further replication is needed.

Several studies have focused on the relationship between basal HPA axis activity and aggressive behavior. However, most of this research has centered on male children and adolescents with externalizing behavior problems. There is evidence that children with externalizing problems display lower basal cortisol levels than their respective control groups, and basal cortisol levels are negatively related to externalizing behavior (Alink et al. 2008). Age seems to be a significant moderator of this relationship, as this pattern was only observed in school-aged children in this meta-analysis. However, children with externalizing disorders not only display enhanced aggressive behavior, but also non-aggressive disruptive behaviors (Hinshaw 1987). Other studies focusing solely on aggressive behavior and basal HPA axis activity have also reported an inverse relationship (McBurnett et al. 2000; Oosterlaan et al. 2005; Pajer et al. 2001; van de Wiel et al. 2004; van Goozen et al. 1998), though some found no relationship (van Bokhoven et al. 2005; van den Bergh et al. 2008). Interestingly, only a few studies on this topic have been conducted in adults. The available findings indicate that habitually violent offenders (Virkkunen 1985) and males with antisocial personality disorder (Bergman and Brismar 1994) have lower basal cortisol levels.

One reason for these divergent results may be methodological differences in the assessment of basal HPA axis activity. Researchers working with humans measure basal HPA axis activity mostly by taking a single measurement of cortisol levels in the morning (Pajer et al. 2001), in the afternoon (Gerra et al. 2001b), or without controlling for time of measurement (McBurnett et al. 2000). To measure trait aspects of HPA axis activity, it is generally better to measure cortisol levels in the morning since they are high (Levine et al. 2007) and more genetically influenced than afternoon or evening levels, which are mostly driven by situational factors (Schreiber et al. 2006; Wüst et al. 2000a). Increasingly, researchers use the cortisol awakening response - a surge in cortisol levels following awakening - as an index of basal HPA axis activity (Kuehner et al. 2007; Marsman et al. 2008; Wirtz et al. 2007). Hellhammer et al. (2007) suggest that to reliably estimate trait components of HPA axis activity, the cortisol awakening response should be assessed with four measurements on at least two consecutive days. Thus, the inconsistent results regarding basal HPA axis activity and aggression may in fact be due to differing situational influences as well as differences in the time of cortisol assessment.

In summary, no clear relationship between basal HPA axis activity and aggressive behavior in humans has emerged, but there is some evidence for an acute increase in HPA axis activity in healthy males following aggression. Although it is important to understand the developmental pathways and the biological markers of aggression in clinical groups, most of the problems for society may result from escalated conflict situations involving healthy individuals (Nelson and Trainor 2007). Therefore, additional studies with healthy male and female subjects are necessary.

The aim of this study was to elucidate the relationship between basal and acute HPA axis activity and aggressive behavior in a group of healthy students. We chose a modified version of the Taylor Aggression Paradigm, also known as the competitive reaction-time task, to induce and measure aggressive behavior in our subjects (Taylor 1967). This paradigm has been extensively validated (Anderson and Bushman 1997; Bernstein et al. 1987; Giancola and Zeichner 1995). We measured the cortisol awakening response on three consecutive days in a group of healthy subjects to obtain a reliable index of trait components of HPA axis activity. In an experimental session, we provoked aggressive behavior with the Taylor Aggression Paradigm and measured acute HPA axis activity. We expected a rise in cortisol levels in the provoked group and, within the provoked group, a relationship between the extent of

aggressive behavior and the rise in cortisol levels. Furthermore, we predicted that basal HPA axis activity would be negatively correlated with aggressive behavior.

2.2 Methods

2.2.1 Subjects

Twenty students of the University of Trier, Germany, (10 female and 10 male, mean age = 23, $SD = 2.7$, range 20-29) took part in the study. All subjects were right-handed. Only non-smokers were included, since smoking is known to affect HPA-axis activity (Granger et al. 2007). Furthermore, they were physically and psychologically healthy with no history of psychiatric disorders. To control for hormonal status in females, only those using hormonal contraceptives were included in the study. The experiment was conducted in accordance with the Declaration of Helsinki. The Research Ethics Committee of the University of Trier approved the study, and all subjects gave written informed consent. Subjects were compensated with 30€ (approximately US \$40).

2.2.2 The Taylor Aggression Paradigm

Aggression was elicited with the Taylor Aggression Paradigm. Subjects were led to believe that they were playing a competitive reaction time task against another subject of the same sex, who they met before the start of the experiment. The game consisted of 30 trials divided into three blocks of 10. In each trial, subjects were instructed to react as quickly as possible to a green square by pressing a key. Subjects were informed that whoever lost a given trial would receive a blast of noise from the winner. Prior to each trial, subjects were directed to select the duration and volume of the noise to be presented to their competitor. Noise duration could be varied between 0 (level 0) and 5 s (level 10) in 0.5 s increments. Volume varied between 60 (level 1) and 105 dB (level 10) in 5 dB increments. Level 0 on the volume scale corresponded to 0 dB. After each trial, feedback about the outcome of the trial was presented on the screen (i.e., whether the subject won or lost). Unknown to the subjects, there was no actual 'competitor'. The outcome of the trials was held constant for all subjects (i.e., each subject won and lost half of the trials). Additionally, noise volume and duration were selected by the experimenter and varied by trial block. During the first block, all subjects received short and gentle noises when they lost a trial (volume: $M = 62.5$ dB, range 0-70 dB; duration: $M = 0.75$ s, range 0-1.5 s). Subjects in the non-provoked control group received the same noises during the second and third block. Subjects in the provoked group received noises of intermediate intensity and duration in the second block (volume: $M = 82.5$ dB, range 75-90

dB; duration: $M = 2.75$ s, range 2-3.5 s) and high intensity and duration in the third block (volume: $M = 99$ dB, range 90-105 dB; duration: $M = 4.4$ s, range 3.5-5 s). The duration and volume settings of the subjects were recorded in each trial on the scales from 0 to 10. An average was computed for each subject and each trial of the volume and duration setting. Finally, the 10 trials belonging to one block of the Taylor Aggression Paradigm were averaged for each subject. These values were later used as the dependent variable "aggressive behavior".

2.2.3 Acute HPA axis activity

During the experiment, salivary cortisol samples were collected once prior to the induction of aggression and three times after. Subjects obtained native saliva in 2-ml reaction tubes (Sarstedt, Nümbrecht, Germany). Collection tubes were positioned on the table in front of the subject and sampling instructions were given via computer. Immediately following the experiment, samples were frozen for biochemical analysis. Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection as described in detail elsewhere (Dressendörfer et al. 1992). Intra- and interassay variability was less than 10 and 12%, respectively.

2.2.4 Basal HPA axis activity

To obtain a reliable trait measure of HPA axis activity, the cortisol awakening response was assessed on three consecutive weekdays prior to the experiment (Hellhammer et al. 2007). Subjects collected samples of native saliva at home each day at awakening and 30, 45, and 60 min later. Awakening time was arranged between 6:00 and 8:00 h for all subjects since awakening time has been shown to influence the cortisol awakening response (Kudielka and Kirschbaum 2003). We tried to choose a time that fitted into the routine of each subject. In addition, time of awakening was held constant intraindividually over the three days. During the sampling period, subjects drank nothing but water and refrained from brushing their teeth, eating, and exercising. The subjects stored all samples in the refrigerator or freezer until returning them to our laboratory on the day of the experiment. These samples were analyzed in the same manner as those obtained during the experiment (see 2.2.3).

We chose to compute the area under the curve with respect to ground (AUC_G) of the cortisol awakening response as a trait measure of HPA axis activity (Hellhammer et al. 2007). AUC_G was calculated by the formula reported in Pruessner et al. (2003) and represents the entire area

under the cortisol awakening response with respect to ground. The AUC_G was calculated for each subject and day and then averaged over the 3 days to form one reliable indicator of basal HPA axis activity for each subject. AUC_G for 19 subjects were included in analysis, 9 in the non-provoked control and 10 in the provoked group. One subject was excluded due to lack of compliance with the sampling schedule on all 3 days. Compliance was defined as a deviation of no more than 10 min from the targeted time for the first and 7 min for the other samples (Kudielka and Kirschbaum 2003). Additionally, the AUC_G s of two subjects (one from each group) were averaged across only 2 days due to non-compliance on the third day. Their data was retained since the reliability of the AUC_G when averaged over 2 days is still acceptable (Hellhammer et al. 2007). Exclusion of these values resulted in correlations of AUC_G across the 3 days between .34 and .65. This is consistent with values reported by others (Wüst et al. 2000b).

2.2.5 Procedure

All subjects were examined individually. We invited the subjects to a preliminary interview, in which we checked the exclusion criteria and informed them of the aim of the study and the procedure. Subjects were told that we wanted to assess the relationship between the steroid hormone cortisol, personality and the perception of and reaction to visual stimuli. The cortisol sampling and experimental procedures were also described. Eligible subjects received sampling devices and a protocol to record sampling times, as well as specific instructions concerning sleep and wake-up times on the night preceding and the morning of the sampling. Participants also received a battery of personality questionnaires to fill out at home. We further emphasized the necessity to adhere to the written instructions and sampling times.

The experiment was conducted between 13:00 and approximately 19:00 h, beginning at 13:00 h, 15:00 h, and 17:00 h, where endogenous cortisol levels are low (Schreiber et al. 2006). The 20 subjects were randomly assigned to the provoked or non-provoked control condition, all the while keeping sex balanced across groups (five males and females in each group). Upon arrival to the laboratory, each subject returned the questionnaires and cortisol samples and was introduced to another subject of the same sex (i.e., a confederate) - with whom he or she was to play a computer game during the experiment. Subjects were then seated in a dimly lit, sound-attenuated room, 1 m from the computer screen. A computer keyboard and the tubes for the collection of salivary cortisol were on a table in front of them.

Each subject was fitted with an EEG-recording device (results reported elsewhere). All instructions were presented via computer. Subjects first gave a salivary cortisol sample (C1, baseline measurement). Next, they completed the Taylor Aggression Paradigm, which lasted for about 10 min. Following the aggression task, subjects gave a second cortisol sample (C2, +15 min after baseline). Finally, all subjects completed a non-stressful task for approximately 20 min and after this gave a third cortisol sample (C3, +35 min) and another one about 10 min later (C4, +45 min). Following completion of the session, all subjects were extensively debriefed, thanked and compensated for their participation. All experimental stimuli were presented and all reactions were recorded with E-Prime© experiment presentation software (Psychological Software Tools, Pittsburgh, PA). The entire laboratory session lasted approximately 90 min.

2.2.6 Statistical analyses

Basal HPA axis activity and aggressive behavior. To examine the effect of the induction of aggression, repeated measures analysis of variance was conducted. Block of the Taylor Aggression Paradigm was entered as the within-subjects factor, group (i.e., provoked vs. non-provoked) was entered as a between-subjects factor and aggressive behavior as the dependent variable. Furthermore, we included gender as a control factor, but for lack of subjects in each cell excluded interactions with other variables in the model. AUC_G of the cortisol awakening response was added as a continuous factor to check for main and interaction effects concerning basal cortisol levels and aggressive behavior. Since the values of the AUC_G were not skewed in the entire sample or in the two groups, the values were not log transformed prior to analysis. However, the covariate AUC_G was z-standardized (Aiken and West 1991).

Acute cortisol levels and aggressive behavior. We performed an ANOVA with the between-subject factor group, the within-subject factor time of cortisol measurement and cortisol level as the dependent variable to check, whether cortisol levels increased in the provoked group due to provocation. Additionally, we controlled for gender and time of experiment (13 h, 15 h, 17 h). Since the salivary cortisol measures taken during the experiment (C1-C4) were slightly skewed, they were log transformed prior to analysis. Since we found differences in our sample with respect to cortisol levels at baseline (C1) in the analysis of variance, we conducted an additional univariate analysis of covariance with the factor group and cortisol levels after the induction of aggression (C3, 20 min after aggression induction) as the dependent variable with cortisol levels at baseline included as a covariate. Additionally, we

correlated aggressive behavior with the change in cortisol levels from before to after the Taylor Aggression Paradigm in the provoked group to analyze whether the amount of aggression in this group was related to an increase in cortisol levels. Since it takes about 20-30 min after a stressor for cortisol levels to reach their peak in saliva (Kirschbaum and Hellhammer 1989), the difference between sample C3 (20 min after induction of aggression) and the baseline sample C1 was calculated. Note that for better interpretation Figure 3 shows original non-transformed cortisol values as the mean \pm standard error of the mean (SEM).

For the ANOVAs, the degrees of freedom were Huynh-Feldt corrected if the assumption of sphericity was violated and only adjusted results are reported (Huynh and Feldt 1976). We calculated Hays' ω^2 as an effect size measure (Hays 1974). An effect of 1% is considered small, 5% is considered medium and 14% is considered a large effect (Cohen 1988). In case of significant effects, we used Dunn's multiple comparison procedure as well as Pearson's correlations as post hoc tests. Statistical analyses were conducted with SPSS for Windows (Version 14.0, SPSS Inc.). The statistical significance level was set to $\alpha = 0.05$.

2.3 Results

2.3.1 Subjects' characteristics

Table 1 shows a comparison of subjects from the provoked and non-provoked control group. Subjects assigned to either condition of the Taylor Aggression Paradigm did not differ in demographic variables, time of experiment, wake-up time, sleep duration, cortisol increase from 0 to 30 min post-awakening, and area under the curve AUC_G of the cortisol awakening response.

Table 1 Characteristics of the subjects in the provoked group and non-provoked control group ($n = 20$)

	Provoked	Non-provoked	Statistical test value	p -Value
	M (%) (SD)	M (%) (SD)		
Age (years)	23.50 (2.87)	22.60 (2.50)	$t(18) = 0.75$.465
% Women	50	50	$\chi^2(18) = 0.00$	1.00
Time of experiment (h)	14:12 (01:24)	15:24 (01:35)	$t(18) = -1.80$.089
Wake-up time (h) ^a	07:04 (00:38)	07:02 (00:33)	$t(17) = 0.12$.905
Sleep duration (h) ^a	06:26 (00:55)	06:36 (01:08)	$t(17) = -0.37$.716
Mean increase (nmol/l) ^{a,b}	5.90 (2.92)	6.19 (3.71)	$t(17) = -.19$.853
AUC_G (nmol/l) ^{a,c}	777.96 (126.81)	752.91 (179.38)	$t(17) = 0.32$.754

^a $n = 19$

^b Mean increase = Mean cortisol increase from 0 to 30 min post awakening

^c AUC_G = Area under the curve with respect to ground of the cortisol awakening response

2.3.2 Manipulation check: aggressive behavior in the Taylor Aggression Paradigm

The means and standard errors for each group and block are presented in Figure 1. The provoked group displayed generally more aggressive behavior ($M = 3.39$, $SEM = 0.30$) than the non-provoked control group ($M = 2.30$, $SEM = 0.31$). This was especially the case for the second and third block of the Taylor Aggression Paradigm. The main effects of group ($F(1,14) = 6.37$, $p = .024$, $\omega^2 = .22$) and of block of the Taylor Aggression Paradigm ($F(2,28) = 13.43$, $p = .000$, $\omega^2 = .30$) were significant and large, as was the interaction group*block of the Taylor Aggression Paradigm ($F(2,28) = 8.40$, $p = .003$, $\omega^2 = .21$). Post-hoc tests showed that the provoked group was significantly more aggressive than the non-provoked control group in block 2 and 3. Furthermore, within the provoked group, all blocks were significantly different from one another, with subjects being least aggressive in block 1 and most aggressive in block 3. The non-provoked control group showed low levels of aggression throughout the experiment.

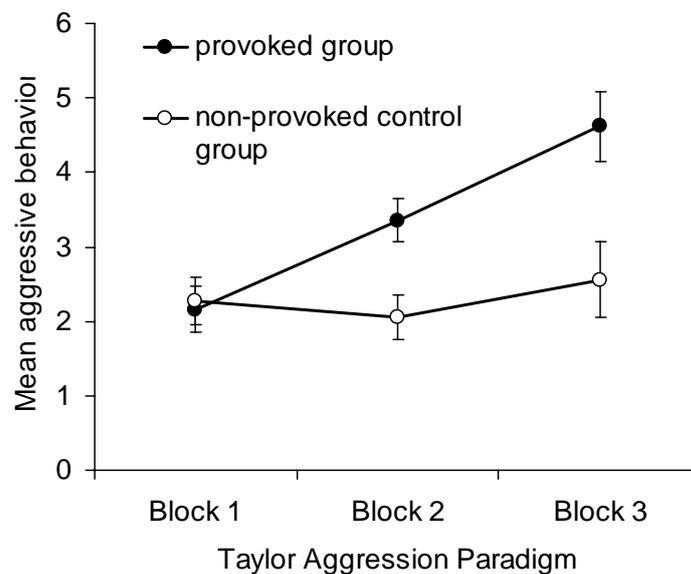


Figure 1 Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the provoked group and the non-provoked control group. Values are means \pm SEM.

2.3.3 Basal cortisol levels and aggressive behavior

The cortisol awakening response was robust on all three days. Cortisol levels increased on average 75% from awakening to 30 min after, which is above average (Wüst et al. 2000b).

The area under the curve of the cortisol awakening response could explain a majority of the variance of aggressive behavior seen in the provoked group. Although there was no main effect of AUC_G ($F(1,14) = 1.04, p = .325$), it interacted significantly with group ($F(1,14) = 14.64, p = .002, \omega^2 = .42$). Post-hoc Pearson correlations revealed a significant negative relationship between AUC_G and aggressive behavior in the provoked group ($r = -.82, p = .003$), displayed in Figure 2. In the non-provoked control group, this correlation was positive, but not significant ($r = .53, p = .144$). No other main or interaction effects with AUC_G were found (all F 's < 2.72 , all p 's $> .098$). The same results were obtained, when AUC_G was log transformed prior to analysis.

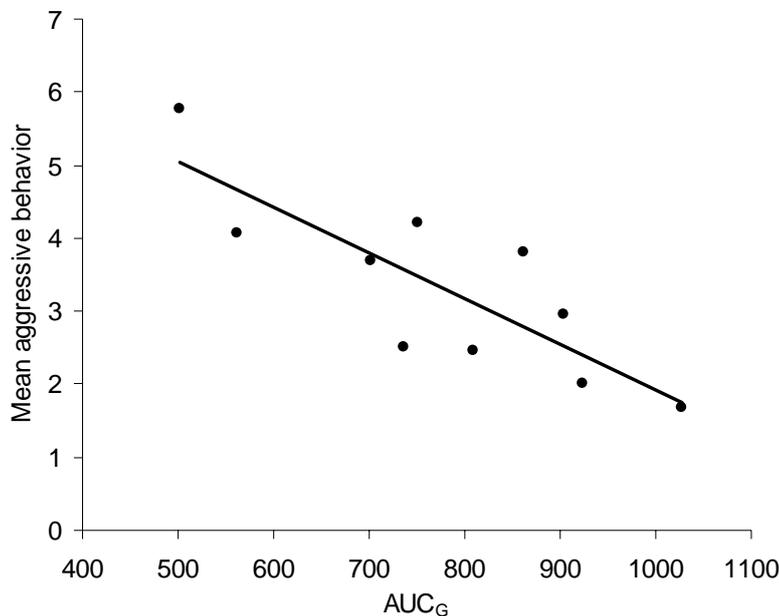


Figure 2 Correlation of the area under the curve AUC_G of the cortisol awakening response and mean aggressive behavior in the provoked group ($n = 10$). $R^2 = .67$.

2.3.4 Acute cortisol levels and aggressive behavior

Cortisol levels only increased slightly in the provoked group. The means and standard errors of the cortisol measurements in both groups are presented in Figure 3. There was a significant interaction effect of time of cortisol measurement and group ($F(3,45) = 4.67, p = .012, \omega^2 = .12$). However, post-hoc tests indicated that the non-provoked control group had higher cortisol levels at baseline and time point C2 than the provoked group. Furthermore, within the non-provoked control group cortisol levels decreased significantly over time from baseline to samples C3 and C4. The increase in the provoked group was not significant. The univariate analysis of covariance comparing the two groups at time point C3, while controlling for baseline levels was, however, highly significant ($F(1,16) = 10.05, p = .006, \omega^2 = .32$), with

higher cortisol levels in the provoked group. Neither the main effect of group ($F(1,16) < 1$), nor the main effect of time of cortisol measurement ($F(3,45) = 1.91, p = .159$) were significant. Furthermore, the difference in cortisol levels in the provoked group from before (C1) to after the Taylor Aggression Paradigm (C3) was not related to the amount of aggressive behavior: neither aggressive behavior averaged over all blocks ($r = .04, p = .911$), nor in any one block ($-0.06 < \text{all } r\text{'s} < .31, \text{all } p\text{'s} > .385$).

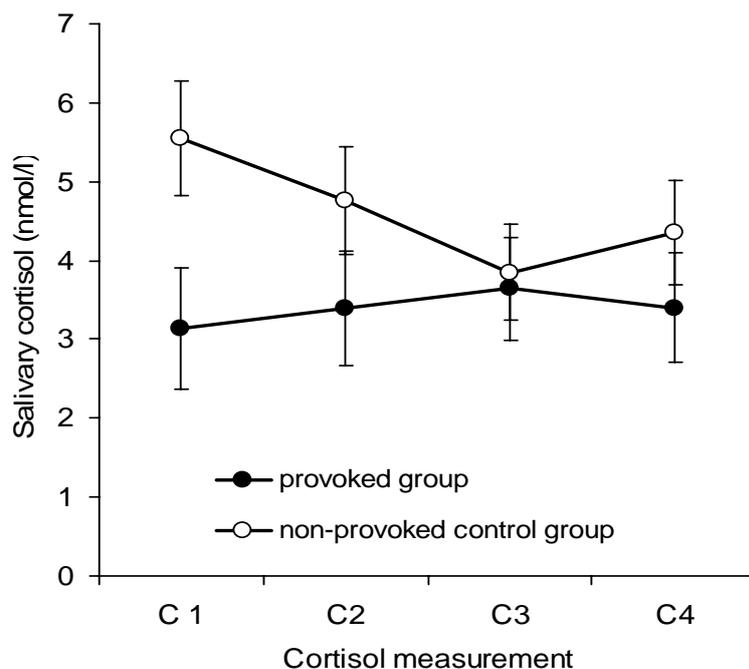


Fig. 3 Cortisol levels during the experiment in the provoked group and the non-provoked control group. C1, baseline before aggression induction; C2, shortly after aggression induction; C3, about 20 min after aggression induction; C4, at the end of the experiment, about 30 min after aggression induction. Values are original means \pm SEM.

2.4 Discussion

The purpose of the present study was to analyze the relationship between aggressive behavior and basal as well as acute HPA axis activity. We experimentally induced aggressive behavior with the Taylor Aggression Paradigm and related the displayed aggressive behavior to basal and acute HPA axis activity levels in healthy subjects. Overall, subjects in the provoked group exhibited more aggressive behavior than the non-provoked controls. There was a significant decrease in cortisol levels in the non-provoked subjects that was absent in the provoked group, leading to significantly higher cortisol levels in the provoked group after the induction of aggression, when baseline cortisol levels were controlled for. Additionally, basal HPA axis activity accounted for a large portion of the variance in aggressive behavior within the

provoked group. In the following section, we will first review the data from the Taylor Aggression Paradigm. We will then discuss the results with respect to the acute levels of HPA axis activity and aggressive behavior, after which we will address the results of basal HPA axis activity and aggressive behavior.

Results indicate that the induction of aggression with the Taylor Aggression Paradigm was successful. Subjects in the provoked group reacted more aggressively during blocks 2 and 3 than those in the non-provoked control group. Aggressive behavior also significantly increased over the three blocks in the provoked group. The interaction of group and block of the Taylor Aggression Paradigm accounted for 21% of the behavioral variance.

The interaction of group and time of cortisol measurement was significant, but this effect was due to differences in baseline cortisol levels and changes within the non-provoked control group. Specifically, cortisol levels in the non-provoked control group were higher at the beginning and decreased significantly over the course of the experiment, whereas cortisol levels in the provoked group remained stable for the entire time. Since participants were randomly assigned to the two groups, the small number of participants may have contributed to this effect. When controlling for these baseline differences in cortisol levels between groups, there was a significant and large difference in cortisol levels after the induction of aggression, with higher levels in the provoked group. Whereas the decrease observed in the non-provoked control group may represent the normal afternoon decline in cortisol levels, the absence of a decrease in cortisol levels in the provoked group may indicate enhanced activity of the HPA axis (Reuter 2002). However, in contrast to other studies (Gerra et al. 2007) we did not find a correlation between aggressive behavior and the amount of cortisol increase. A reason for these discrepant results could be our choice of the Taylor Aggression Paradigm to induce aggression. The Taylor Aggression Paradigm was shorter in duration than the task used by Gerra et al. and might as such have been less stressful. This is supported by another study that used the Taylor Aggression Paradigm and assessed acute HPA axis activity (Berman et al. 1993), which also found no increase in cortisol levels in the provoked group.

Basal HPA axis activity was significantly and negatively related to aggressive behavior in the provoked group and accounted for as much as 67% of the variance in aggressive behavior across all blocks. Subjects with lower levels of basal HPA axis activity chose higher and longer noise settings for their opponents upon being provoked. As demonstrated in a study

with animals, chronically low basal glucocorticoid levels have been linked to extreme forms of aggression. For example, Halasz et al. (Halasz et al. 2002) showed that glucocorticoid deficiency was associated with changes in neural functioning, including a heightened activation of the central amygdala. This might lead to social deficits, where ambiguous or neutral situations are misinterpreted and thus lead to a lower threshold for aggressive behavior. Indeed, changes in social information processing, especially of ambiguous social stimuli, have been documented in highly aggressive children (Milich and Dodge 1984). We speculate that in our healthy subjects the same mechanisms might be involved, only in a less severe manner. Subjects with low basal HPA axis activity may be more sensitive to situational provocation and react more aggressively than subjects with higher basal HPA axis activity. In contrast, this negative relationship between aggressive behavior and basal HPA axis activity was not present in the non-provoked control group, where the correlation was positive, albeit non-significant. Qualitative differences between aggressive behavior in the two groups may explain these different associations with basal HPA axis activity, since aggressive behavior in the provoked group was more of a reactive kind compared to that in the non-provoked group, which was also generally lower (Bettencourt et al. 2006). Several studies have noted different underlying biological mechanisms for these aggression subtypes (Nelson and Trainor 2007; Strüber et al. 2008). This has also been recently shown for the HPA axis (Lopez-Duran et al. 2009), in that HPA axis reactivity to stressful situations in children was positively related to reactive, but not to proactive aggression. By extension, it seems likely that low basal HPA axis activity may lead to heightened reactive aggression in situations involving provocation, but may impede the elicitation of (proactive) aggression in non- or only low provoking situations. However, the positive correlation for proactive aggression displayed by the non-provoked control group needs to be validated with a larger sample.

When interpreting the results of the present study, several limitations have to be kept in mind. First, we chose to analyze a relatively small number of subjects. However, even with this small sample, a significant and large effect of basal HPA axis activity and aggression was observed in the provoked group. Second, we could not analyze the interaction effects of sex with other independent variables since the amount of subjects in each cell was too low. But, we controlled for sex effects by having an equal amount of men and women in each group and by including this factor as a covariate into the analysis. Third, as discussed above, the provocation we used was relatively mild compared to other experimental settings, which have

previously been used to activate the HPA axis, or real world conflict situations. While this may explain our lack of findings for an increase in cortisol, we did find a significant difference between the two groups, in that the provoked group had higher levels than the non-provoked control group when controlling for baseline levels. Furthermore, the significant differences observed in aggressive behavior between the provoked and non-provoked control group suggest that the task was effective.

This is the first time that the relationship between trait components of HPA axis activity and aggressive behavior was analyzed experimentally in a group of healthy subjects. When trait aspects of basal HPA axis activity are reliably assessed, large effects on aggressive behavior within healthy adults can be observed in provoking situations. The study further underlines the importance of distinguishing between basal and acute HPA axis activity, since both relate to aggressive behavior in different ways, and of measuring them accordingly.

2.i Acknowledgments

This research was supported by the International Research Training Group "The Psychoneuroendocrinology of Stress" of the Deutsche Forschungsgemeinschaft (DFG, GRK1389/1, Project H). The authors wish to thank John J. Sollers III and LaBarron Hill from The Ohio State University for language editing of the manuscript.

2.ii References

- Aiken LS, West SG (1991) Multiple regression: testing and interpreting interactions. Sage, Newbury
- Alink LR, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Mesman J, Juffer F, Koot HM (2008) Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev Psychobiol* 50: 427-450
- Anderson CA, Bushman BJ (1997) External Validity of "Trivial" Experiments: the Case of Laboratory Aggression. *Review of General Psychology* 1: 19-41
- Baron RA, Richardson DR (1994) Human Aggression. Plenum Press, New York
- Bergman B, Brismar B (1994) Hormone levels and personality traits in abusive and suicidal male alcoholics. *Alcohol Clin Exp Res* 18: 311-316
- Berman M, Gladue B, Taylor S (1993) The effects of hormones, Type A behavior pattern, and provocation on aggression in men. *Motivation and Emotion* 17: 125-138
- Bernstein S, Richardson D, Hammock G (1987) Convergent and discriminant validity of the Taylor and Buss measures of physical aggression. *Aggressive Behavior* 13: 15-24
- Bettencourt BA, Talley A, Benjamin AJ, Valentine J (2006) Personality and aggressive behavior under provoking and neutral conditions: a meta-analytic review. *Psychol Bull* 132: 751-777
- Cohen J (1988) Statistical power analysis for the behavioral sciences. Erlbaum, Hillsdale
- Coryell W, Schlessler M (2001) The dexamethasone suppression test and suicide prediction. *Am J Psychiatry* 158: 748-753
- Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ (1992) Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *J Steroid Biochem Mol Biol* 43: 683-692
- Gerra G, Zaimovic A, Ampollini R, Giusti F, Delsignore R, Raggi MA, Laviola G, Macchia T, Brambilla F (2001b) Experimentally induced aggressive behavior in subjects with

- 3,4-methylenedioxy-methamphetamine ("Ecstasy") use history: psychobiological correlates. *J Subst Abuse* 13: 471-491
- Gerra G, Zaimovic A, Avanzini P, Chittolini B, Giucastro G, Caccavari R, Palladino M, Maestri D, Monica C, Delsignore R, Brambilla F (1997) Neurotransmitter-neuroendocrine responses to experimentally induced aggression in humans: influence of personality variable. *Psychiatry Res* 66: 33-43
- Gerra G, Zaimovic A, Moi G, Bussandri M, Bubici C, Mossini M, Raggi MA, Brambilla F (2004) Aggressive responding in abstinent heroin addicts: neuroendocrine and personality correlates. *Prog Neuropsychopharmacol Biol Psychiatry* 28: 129-139
- Gerra G, Zaimovic A, Raggi MA, Giusti F, Delsignore R, Bertacca S, Brambilla F (2001a) Aggressive responding of male heroin addicts under methadone treatment: psychometric and neuroendocrine correlates. *Drug Alcohol Depend* 65: 85-95
- Gerra G, Zaimovic A, Raggi MA, Moi G, Branchi B, Moroni M, Brambilla F (2007) Experimentally induced aggressiveness in heroin-dependent patients treated with buprenorphine: comparison of patients receiving methadone and healthy subjects. *Psychiatry Res* 149: 201-213
- Giancola PR, Zeichner A (1995) Construct validity of a competitive reaction-time aggression paradigm. *Aggressive Behavior* 21: 199-204
- Granger DA, Blair C, Willoughby M, Kivlighan KT, Hibel LC, Fortunato CK, Wiegand LE (2007) Individual differences in salivary cortisol and alpha-amylase in mothers and their infants: relation to tobacco smoke exposure. *Dev Psychobiol* 49: 692-701
- Halasz J, Liposits Z, Kruk MR, Haller J (2002) Neural background of glucocorticoid dysfunction-induced abnormal aggression in rats: involvement of fear- and stress-related structures. *Eur J Neurosci* 15: 561-569
- Haller J, Halasz J, Mikics E, Kruk MR (2004) Chronic glucocorticoid deficiency-induced abnormal aggression, autonomic hypoarousal, and social deficit in rats. *J Neuroendocrinol* 16: 550-557
- Hays WL (1974) *Statistics*. Holt, Rinehart & Winston, London

- Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D (2007) Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. *Psychoneuroendocrinology* 32: 80-86
- Hennig J, Reuter M, Netter P, Burk C, Landt O (2005) Two types of aggression are differentially related to serotonergic activity and the A779C TPH polymorphism. *Behav Neurosci* 119: 16-25
- Hinshaw SP (1987) On the distinction between attentional deficits/hyperactivity and conduct problems/aggression in child psychopathology. *Psychol Bull* 101: 443-463
- Huynh H, Feldt LS (1976) Estimation of the box correction for degrees of freedom from sample data in randomized block and splitsplot designs. *Journal of Educational Statistics* 1: 69-82
- Kirschbaum C, Hellhammer DH (1989) Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 22: 150-169
- Kruk MR, Halasz J, Meelis W, Haller J (2004) Fast positive feedback between the adrenocortical stress response and a brain mechanism involved in aggressive behavior. *Behav Neurosci* 118: 1062-1070
- Kudielka BM, Kirschbaum C (2003) Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 28: 35-47
- Kuehner C, Holzhauser S, Huffziger S (2007) Decreased cortisol response to awakening is associated with cognitive vulnerability to depression in a nonclinical sample of young adults. *Psychoneuroendocrinology* 32: 199-209
- Levine A, Zagoory-Sharon O, Feldman R, Lewis JG, Weller A (2007) Measuring cortisol in human psychobiological studies. *Physiol Behav* 90: 43-53
- Lopez-Duran NL, Olson SL, Hajal NJ, Felt BT, Vazquez DM (2009) Hypothalamic pituitary adrenal axis functioning in reactive and proactive aggression in children. *J Abnorm Child Psychol* 37: 169-182
- Marsman R, Swinkels SH, Rosmalen JG, Oldehinkel AJ, Ormel J, Buitelaar JK (2008) HPA-axis activity and externalizing behavior problems in early adolescents from the
-

- general population: the role of comorbidity and gender The TRAILS study. *Psychoneuroendocrinology* 33: 789-798
- McBurnett K, Lahey BB, Rathouz PJ, Loeber R (2000) Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Arch Gen Psychiatry* 57: 38-43
- Milich R, Dodge KA (1984) Social information processing in child psychiatric populations. *J Abnorm Child Psychol* 12: 471-489
- Nelson RJ, Trainor BC (2007) Neural mechanisms of aggression. *Nat Rev Neurosci* 8: 536-546
- Oosterlaan J, Geurts HM, Knol DL, Sergeant JA (2005) Low basal salivary cortisol is associated with teacher-reported symptoms of conduct disorder. *Psychiatry Res* 134: 1-10
- Pajer K, Gardner W, Rubin RT, Perel J, Neal S (2001) Decreased cortisol levels in adolescent girls with conduct disorder. *Arch Gen Psychiatry* 58: 297-302
- Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003) Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28: 916-931
- Reuter M (2002) Impact of cortisol on emotions under stress and nonstress conditions: a pharmacopsychological approach. *Neuropsychobiology* 46: 41-48
- Schreiber JE, Shirtcliff E, Van Hulle C, Lemery-Chalfant K, Klein MH, Kalin NH, Essex MJ, Goldsmith HH (2006) Environmental influences on family similarity in afternoon cortisol levels: twin and parent-offspring designs. *Psychoneuroendocrinology* 31: 1131-1137
- Strüber D, Lück M, Roth G (2008) Sex, aggression and impulse control: an integrative account. *Neurocase* 14: 93-121
- Taylor SP (1967) Aggressive behavior and physiological arousal as a function of provocation and the tendency to inhibit aggression. *J Pers* 35: 297-310

- van Bokhoven I, Van Goozen SH, van Engeland H, Schaal B, Arseneault L, Seguin JR, Nagin DS, Vitaro F, Tremblay RE (2005) Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *J Neural Transm* 112: 1083-1096
- van de Wiel NM, van Goozen SH, Matthys W, Snoek H, van Engeland H (2004) Cortisol and treatment effect in children with disruptive behavior disorders: a preliminary study. *J Am Acad Child Adolesc Psychiatry* 43: 1011-1018
- van den Bergh BR, van Calster B, Pinna Puissant S, van Huffel S (2008) Self-reported symptoms of depressed mood, trait anxiety and aggressive behavior in post-pubertal adolescents: Associations with diurnal cortisol profiles. *Horm Behav* 54: 253-257
- van Goozen SH, Matthys W, Cohen-Kettenis PT, Gispen-de Wied C, Wiegant VM, van Engeland H (1998) Salivary cortisol and cardiovascular activity during stress in oppositional-defiant disorder boys and normal controls. *Biol Psychiatry* 43: 531-539
- Virkkunen M (1985) Urinary free cortisol secretion in habitually violent offenders. *Acta Psychiatr Scand* 72: 40-44
- Wirtz PH, von Kanel R, Emini L, Ruedisueli K, Groessbauer S, Maercker A, Ehlert U (2007) Evidence for altered hypothalamus-pituitary-adrenal axis functioning in systemic hypertension: blunted cortisol response to awakening and lower negative feedback sensitivity. *Psychoneuroendocrinology* 32: 430-436
- Wüst S, Federenko I, Hellhammer DH, Kirschbaum C (2000a) Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 25: 707-720
- Wüst S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C (2000b) The cortisol awakening response - normal values and confounds. *Noise Health* 2: 79-88

Chapter III

Exogenous cortisol enhances aggressive behavior in females, but not in males

(Böhnke, et al., 2010)

Co-Authors: Katja Bertsch, Menno R. Kruk, Steffen Richter & Ewald Naumann.

3.0 Abstract

The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the development, elicitation, and enhancement of aggressive behavior in animals. Increasing evidence suggests that this is also true for humans. Here, we report on a study of the role of basal and acute HPA axis activity in a sample of 48 healthy male and female adults. We pharmacologically enhanced cortisol levels and used the Taylor Aggression Paradigm (TAP) to induce and measure aggression (divided into three blocks). Participants either received an oral dose of 20 mg hydrocortisone (cortisol group) or a placebo (placebo group). Half of each group received high or low levels of provocation with the TAP, respectively. Before, we assessed the cortisol awakening response as a trait measure of basal HPA axis activity. Participants in the cortisol group reacted more aggressively in the third block of the TAP compared to the placebo group. Furthermore, gender interacted with treatment: only females, but not males showed enhanced aggressive behavior after cortisol administration. There was no significant difference in males between the placebo and cortisol group. Basal HPA axis activity was negatively related to aggressive behavior, but again only in females and most strongly within the placebo group. This study provides the first evidence for a causal involvement of acute HPA axis activation in aggressive behavior in humans.

Keywords

Aggression; Taylor Aggression Paradigm; HPA axis; Cortisol; Cortisol awakening response; Gender differences

3.1 Introduction

Aggressive behavior is a natural and adaptive phenomenon, but it can be problematic for society if it is exaggerated, persistent, or expressed out of context (Nelson and Trainor, 2007). A system believed to be involved in aggressive behavior is the hypothalamic-pituitary-adrenal (HPA) axis and especially its endproduct, the glucocorticoids. In animals, there is extensive evidence for the importance of this system for the development, the elicitation, and the enhancement of aggressive behavior, as well as its role in pathological forms of aggression (Wommack and Delville, 2007). An acute dose of corticosterone lowers the threshold for attack behavior in rats, evoking aggressive behavior more readily (Kruk et al., 2004). Apart from acute increases in cortisol, basal levels of HPA axis activity also seem to be relevant in determining aggressive behavior in animals. An experimentally induced chronic glucocorticoid deficiency is causally involved in heightened aggressive behavior in rats,

leading to abnormal attack behavior of the animal (Haller et al., 2001; Haller et al., 2004; Haller and Kruk, 2006). However, basal HPA axis activity triggers abnormal aggressive behavior only when no acute activation of the HPA axis takes place (Haller et al., 2001). In sum, both acute and basal HPA axis activity causally influence the expression of aggressive behavior, albeit in different ways.

In humans, the HPA axis has been recognized as an important system involved in the development and expression of antisocial behavior, especially in children (van Goozen and Fairchild, 2006; van Goozen et al., 2007). In the following, we will refer to cortisol levels at a specific time point as acute HPA axis activity, trait like aspects of cortisol levels at rest as basal HPA axis activity, and the change in HPA axis activity following a challenge as HPA axis reactivity.

Correlative as well as experimental evidence suggests that acute HPA axis activity is associated with aggressive behavior. In fact, a hyperreactive HPA axis has been postulated to be involved in aggressive behavior (Haller and Kruk, 2006). This has recently been confirmed by a study in humans, where HPA axis reactivity to stressful situations in children was positively related to reactive, but not to proactive aggression as measured with a questionnaire (Lopez-Duran et al., 2009). In a series of experiments, Gerra and colleagues (2007) measured acute HPA axis activity prior to an aggression induction procedure in a group of healthy participants and found cortisol levels to be positively associated with aggressive behavior. Not all studies, however, found the latter effect (Berman et al., 1993; Salvador et al., 1999). Moreover, Verona and colleagues could show that stressful situations also have an impact on aggressive behavior (2006a, 2006b, 2007b). They physically stressed their participants (healthy adult males and females) and concurrently measured levels of aggressive behavior in a teacher-learner paradigm. The stressor seems to enhance aggressive behavior in males, but not in females. However, this could not always be replicated (Verona et al., 2007a).

Basal HPA axis activity is not only important in the aggressive behavior of child-aged clinical groups, but also in healthy adult humans. Most of the studies, have been correlative of nature, mostly using male child and adolescent clinical groups with extreme levels of aggressive behavior. When including the studies with healthy samples, on average an effect of $d = -.40$ can be observed (van Goozen et al., 2007): Low basal cortisol levels were related to enhanced aggressive behavior, mirroring the results of the animal studies. However, another meta-

analysis relating externalizing behavior, which includes aggressive behavior, with cortisol levels could show that this effect was age dependent and only apparent in the group of school-aged children (Alink et al., 2008). In a prior study that we conducted with healthy adult males and females, basal HPA axis activity was negatively related to aggressive behavior and explained more than half of the variance in observed aggressive behavior (R. Böhnke, K. Bertsch, M.R. Kruk, and E. Naumann, unpublished observations).

To summarize, there is evidence that acute as well as basal HPA axis activity play a role in aggressive behavior. However, especially in healthy adults, experimental evidence involving acute and basal HPA axis activity and aggression is rare. Aggressive behavior is not only displayed by humans with different psychopathological conditions, but frequently also by otherwise healthy adults in escalated conflict situations (Nelson and Trainor, 2007). Therefore, it is important to experimentally analyze the relationship of the HPA axis and aggression in healthy individuals, as well.

Also, when studying aggression, gender has to be taken into account, since males are generally more aggressive than females (Bettencourt and Miller, 1996; Anderson and Bushman, 2002). This is especially important when studying the biology of aggression, since at least some biological systems seem to have a different functionality with regard to aggression in males and females (Strüber et al., 2008). However, most of the previously mentioned studies were conducted with male participants only, making it difficult to assess gender specific influences of HPA axis activity. Therefore, we included male and female participants in our study.

The aim of this study was to elucidate the relationship of basal and acute HPA axis activity and aggressive behavior in healthy male and female adults. We chose a modified version of the Taylor Aggression Paradigm, also known as the competitive reaction-time task, to induce and measure aggressive behavior in our participants (Taylor, 1967). This paradigm has been extensively validated and has been shown to have high reliability and external and construct validity for men and women (Bernstein et al., 1987; Giancola and Zeichner, 1995; Anderson and Bushman, 1997; Giancola and Parrott, 2008). Before implementing the Taylor Aggression Paradigm we gave participants either 20mg of hydrocortisone (cortisol group) or a placebo (placebo group) in a double-blind fashion. Half of each group received low levels of provocation (low provocation group), the other half received high levels of provocation (high provocation group) in the Taylor Aggression Paradigm. During the task, the participants in

both groups had the chance to respond aggressively. To reliably assess trait components of basal HPA axis activity, we measured the cortisol awakening response on three consecutive days before the experimental session (Hellhammer et al., 2007). We expected an increase in aggressive behavior in the cortisol group, especially in those receiving high levels of provocation. Additionally, we expected that acute cortisol levels in the placebo group should be positively related to aggressive behavior, especially in those subjects that were highly provoked. Since the literature is inconsistent, we did not have specific hypotheses with respect to gender. Since animal studies showed that low basal HPA axis activity only had an effect on aggressive behavior when the HPA axis was not acutely activated, we expected a relationship of basal HPA axis activity and aggression in the high as well as the low provocation group, but only in those participants that received a placebo. This should apply to males as well as females.

3.2 Methods

3.2.1 Participants

56 students of the University of Trier, Germany, (28 female and 28 male, mean age = 23, $SD = 2.5$, range 19-32) took part in the study. However, data of only 48 participants are reported (24 female and 24 male, mean age = 23, $SD = 2.7$, range 19-32), since eight participants had to be removed from analysis due to reasons discussed in section 2.7¹. Exclusion criteria were as follows: any acute or chronic disease, a presence or history of mental illness and use of medication. Furthermore, since cigarette smoking is known to change HPA axis activity, only non-smokers were included (Granger et al., 2007). Since we gave a standardized dose of hydrocortisone, we only included subjects with a BMI between 19 and 25. Since we also measured the EEG, we had to excluded left-handed subjects, since handedness is related to brain activity. In the Taylor Aggression Paradigm, we used colored stimuli for the reaction time task. Therefore participants were checked for color-blindness in the interview, and were excluded, if they could not distinguish colors. Only non-pregnant women who used hormonal contraceptives were included in the study to control for hormonal status. However, women using the oral contraceptives Yasmine, Yasminelle and Petibelle were excluded, since these medications have the component Drospirenone, which is an antagonist for the mineralocorticoid receptor and therefore might have interfered with the effect of cortisol

¹ To make sure that exclusion of the subjects did not lead to a selection bias, excluded subjects ($N = 8$) were compared to included subjects ($N = 48$) on basal cortisol levels, cortisol levels during the experiment, and aggressive behavior within each block. In all cases, the means of the excluded group were within one standard deviation of the means of the included group.

administration (Genazzani et al., 2007). Medical exclusion criteria were assessed by a physician. The experiment was conducted in accordance with the Declaration of Helsinki. The Ethical Committee of the State's Medical Association (Landesärztekammer Rheinland-Pfalz) approved the study, and all participants gave written informed consent. Participants were compensated with €40 (approximately US \$55).

3.2.2 The Taylor Aggression Paradigm

Aggression was elicited with the Taylor Aggression Paradigm in half of the participants (high provocation group) whereas the other half was only mildly provoked (low provocation group). Participants were led to believe that they were playing a competitive reaction time task against another participant, who they met before the start of the experiment. The game consisted of 30 trials divided up into three blocks of 10. The number of trials was chosen to be as similar as possible to previous studies on the validity of the paradigm (Giancola and Parrott, 2008). The task of the participants in each trial was to react as fast as possible to a green square by pressing a key. They were told that whoever lost a given trial would be presented with a blast of noise by the other person. Prior to every trial, the participants had to set the duration and the volume of the noise for the other person on two separate scales reaching both from 0 to 10. Corresponding to the 11 levels, the duration could be varied between 0 (level 0) and 5 seconds (level 10) in 0.5 second increments. The volume varied between 60 (level 1) and 105 dB (level 10) in 5 dB increments. The level 0 on the volume scale corresponded to 0 dB. After each trial, feedback about the outcome of the trial was presented on the screen, i.e. whether the participant won or lost. In fact, there was no other participant and the outcome of the trials was held constant for all participants - they won and lost half of the trials each. The experimenter also set in advance the volume and duration of the noise according to the block and condition of the participant. During the first block, all participants received short and gentle noises when they lost a trial (volume: $M = 62.5$ dB, range 0-70 dB; duration: $M = 0.75$ s, range 0-1.5 s). Participants of the low provocation group received the same noises during the second and third block, as well. Participants of the high provocation group were exposed to noises of intermediate intensity and duration in the second block (volume: $M = 82.5$ dB, range 75-90 dB; duration: $M = 2.75$ s, range 2-3.5 s) and high intensity and duration in the third block (volume: $M = 99$ dB, range 90-105 dB; duration: $M = 4.4$ s, range 3.5-5 s) whenever they lost a trial. The duration and volume settings of the participants were recorded and an average was computed of the volume and duration setting for each participant and trial, except for when one of the settings was 0. In that case, the total

score was also set to 0 since no noise would have been presented to the other participant and would not have constituted an aggressive act. Finally, the 10 trials belonging to one block of the Taylor Aggression Paradigm were averaged for each participant. These values were later used as the dependent variable "aggressive behavior" in statistical analysis and can also be seen in the figures.

3.2.3 Feelings of anger and negative affect

To validate that the Taylor Aggression Paradigm did in fact lead to more negative and especially angry feelings, we administered the German version of the state Positive and Negative Affect Schedule (PANAS; Krohne et al., 1996) before and after the aggression induction procedure.

3.2.4 Acute HPA axis activity

To acutely enhance circulating cortisol levels, half of the participants of both the high and low provocation group received an oral dose of 20mg of hydrocortisone (cortisol group) 1 h prior to the aggression induction. The other half were administered a placebo (placebo group). In the debriefing session, the participants' feeling of whether cortisol or placebo was administered, was inquired.

Salivary cortisol samples were collected once directly before the administration of hydrocortisone/placebo, and during the experimental session once before the induction of aggression and three times after. Participants obtained native saliva in 2 ml reaction tubes (Sarstedt, Nümbrecht, Germany). The first sample was taken in the waiting room and participants were instructed by the experimenter, the other samples were taken in the laboratory. There, the tubes for collection were stationed on the table in front of the participant and the instructions were given via the computer. Directly after the experiment, the samples were frozen for biochemical analysis. Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection as described in detail elsewhere (Dressendörfer et al., 1992). Intra- and interassay variability was 4,0 - 6,7% and 7.1 - 9.0%, respectively. For the participants who were administered hydrocortisone, a cut-off was set at 100 nmol/l for the samples taken after hydrocortisone administration (samples C2-C5), since the assay did not permit reliable measurements above 100 nmol/l.

3.2.5 Basal HPA axis activity

To get a reliable trait measure of HPA axis activity, the cortisol awakening response was assessed on three consecutive weekdays prior to the experiment (Hellhammer et al., 2007). The participants collected samples of native saliva at home each day at awakening and 30, 45, and 60 minutes later. Awakening time was arranged between 6:00 and 8:00 h for all participants since awakening time has been shown to influence the cortisol awakening response (Kudielka and Kirschbaum, 2003). We tried to choose a time that fitted into the routine of each participant. In addition, time of awakening was held constant intraindividually over the three days. During the sampling period, participants drank nothing but water and refrained from brushing their teeth, eating, and exercising. The participants stored all samples in the refrigerator or freezer until returning them to our laboratory on the day of the experiment. The samples were processed in the same way as the samples collected during the experiment (see 2.4). We computed the area under the curve with respect to ground (AUC_G) of the cortisol awakening response as a trait measure of HPA axis activity (Hellhammer et al., 2007). It was calculated by the formula reported in Pruessner et al. (2003) and represents the entire area under the cortisol awakening response with respect to ground. The AUC_G was calculated for each participant and day and then averaged over the three days to form one reliable indicator of basal HPA axis activity for each participant. The correlations of the AUC_G of the three days for the 48 included participants ranged between $r = .68$ and $r = .72$, which is above values reported by others (Wüst et al., 2000b).

3.2.6 Procedure

All participants were examined individually. We invited the participants to a preliminary interview, in which we checked the exclusion criteria and informed them about the aim of the study and the procedure. In the preliminary interview, participants were told that we wanted to assess the relationship between the steroid hormone cortisol and the perception of and reaction to visual stimuli. We informed them about the cortisol sampling and the experimental procedure. Then the dates and times for the sampling of the cortisol awakening response and the experiment were arranged. Finally, they received a battery of personality questionnaires to fill out at home. They also took home sampling devices for salivary cortisol and a protocol to keep records of the time of sampling, the time of going to sleep the previous night, and wake-up time on the morning of the sampling. We further emphasized the necessity to adhere to the written instructions and sampling times. In a separate meeting, a physician conducted a

clinical interview with the participants and checked the health status and medical exclusion criteria.

The experiment was conducted between 12:00 and approximately 19:20 h, beginning at 12:00 h, 14:30 h and 17:00 h, where endogenous cortisol levels are low (Schreiber et al., 2006). The originally 56 participants were randomly assigned to the provoked or non-provoked control condition and the cortisol and placebo groups, but sex was balanced across groups. Of the 48 participants analyzed, 11 were in the high provocation/cortisol, 11 were in the high provocation/placebo group, 13 were in the low provocation/cortisol, and 13 were in the low provocation/placebo group. On arrival, we acquainted the participant with another participant of the same sex - in fact, a confederate of the investigator - with whom he or she was to play a computer game during the experiment. The participant as well as the confederate handed over the filled out questionnaires and the salivary cortisol samples they collected at home. Participants were seated in a waiting room, where they gave the baseline cortisol sample (C1, baseline, -1 h). Then, they were administered 20mg of hydrocortisone or a placebo. They were then left alone in the room to fill out questionnaires. The confederate was ostensibly seated in a different room. After 30 min, the experimenter escorted the participant to the room in which the experimental session took place. They were seated in a dimly lit sound-attenuated room, 1 m from the computer screen. A computer keyboard and the tubes for the collection of salivary cortisol were on a table in front of them.

The participants were fitted with an EEG and ECG-recording device and were left alone in the room for the remainder of the experiment. They received instructions via the computer screen. The computer program was started exactly 1 h after the administration of hydrocortisone/placebo. First, they gave a salivary cortisol sample (C2, 0 min) and filled out the PANAS state questionnaire. Then, they played the Taylor Aggression Paradigm, which lasted for about ten minutes. Afterwards, they gave a third cortisol sample (C3, +15 min) and filled out the PANAS state again. They worked on an emotional Stroop task for about 20 minutes (results reported elsewhere), gave their fourth (C4, + 40 min) and fifth (C5, +50 min) cortisol sample, and were extensively debriefed. We thanked and compensated them for their participation. Stimuli were presented and the reactions were recorded with E-Prime presentation software (Psychological Software Tools, Pittsburgh, PA). The experiment, from arrival to debriefing had a duration of about 140 minutes. Figure 4 presents a timeline of the experimental procedure.

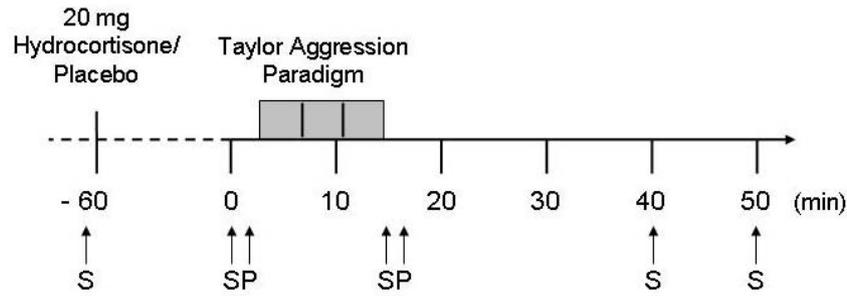


Figure 4 Timeline of the experiment. S = Salivary cortisol sample; P = PANAS state questionnaire.

3.2.7 Statistical analyses

Of the 56 participants, only 48 could be included in the statistical analyses. One subject (high provocation/cortisol group) had outlier values on all three blocks of the Taylor Aggression Paradigm, in that he responded with extremely high aggression. One subject (high provocation/cortisol group) did not respond with elevated salivary cortisol levels at C2 (1 h after oral administration) but only towards the end of the entire session (C5) and was therefore removed. Six subjects did not comply with the protocol for ambulant salivary cortisol assessment on two or three days (two each in the high provocation/cortisol and high provocation/placebo group, and one each in the two low provocation groups). Compliance was defined as a deviation of more than 10 minutes from the targeted time for the first, and 7 minutes for the other samples (Kudielka and Kirschbaum, 2003). These subjects were removed from all of the analysis, i.e. results of the same 48 subjects are reported for the different analyses.

To validate the Taylor Aggression Paradigm with the PANAS, we conducted 2x2x2 analysis of variance with provocation group (high and low provocation), treatment (cortisol and placebo), and measurement time (before vs. after the Taylor Aggression Paradigm) for the negative affect scale of the PANAS and for the item "feelings of anger". To check, whether the aggressive behavior was related to the amount of provocation, the acute increase in cortisol levels, basal cortisol levels or gender, we calculated an analysis of variance with provocation group, treatment (cortisol and placebo), and gender as a between-subject factor, Block of the Taylor Aggression Paradigm as a within-subjects factor, and aggressive behavior as the dependent variable. The area under the curve with respect to ground AUC_G of the cortisol awakening response was added as a continuous factor to check for main and interaction effects concerning basal cortisol levels and aggressive behavior. Since the values of the AUC_G were not skewed in the entire sample or in the two groups, the values were not

log transformed prior to analysis. However, the continuous factor AUC_G was z-standardized (Aiken and West, 1991). Additionally, to further check whether acute cortisol levels were related to aggressive behavior, the cortisol levels during the experiment and were correlated with aggressive behavior within the placebo group. Since cortisol levels within the placebo group did not change much over the experiment (increase of 0.66 nmol/l), the log transformed data were averaged over all five samples and were correlated with aggressive behavior in each block and mean aggressive behavior. Since cortisol is released in a diurnal fashion, we checked whether the time of the experiment was related to cortisol levels before, during, or after the Taylor Aggression Paradigm. Since the correlations were close to zero ($-.051 < r's < -.092$), time of the experiment was not added as an additional variable in the analyses.

The degrees of freedom were Huynh-Feldt corrected if the assumption of Sphericity was violated and only adjusted results are reported (Huynh and Feldt, 1976). We calculated Hays' ω^2 as an effect size measure (Hays, 1974). An effect of 1% is considered small, 5% is considered medium and 14% is considered a large effect (Cohen, 1988). For the analysis, only significant effects of at least $\omega^2 = 5\%$ were deemed relevant and are reported. Where appropriate, we used Dunn's Multiple Comparison Procedure as well as Pearson correlations as post-hoc tests. Statistical analyses were conducted with SPSS for Windows (Version 17.0, SPSS Inc.). The statistical significance level was set to $\alpha = 0.05$.

3.3 Results

3.3.1 Participants' characteristics and manipulation check

Table 2 shows a comparison of participants from the four experimental groups. Participants assigned to either condition of the Taylor Aggression Paradigm and cortisol or placebo administration did not differ in demographic variables, time of experiment, wake-up time, sleep duration, area under the curve AUC_G of the cortisol awakening response, and baseline cortisol measurement (C1). However, we found slightly positive associations between basal cortisol levels and wake-up time for the three days ($r = .13$ to $.35$) and overall wake-up time and AUC of the cortisol awakening response ($r = .31$). Participants in the entire placebo group differed significantly from participants in the cortisol group with respect to log transformed cortisol levels at the start of the Taylor Aggression Paradigm (C2), indicating that the acute enhancement of cortisol levels with hydrocortisone was successful. The participants in the cortisol group had original cortisol levels well above those of the placebo group before (cortisol: $M = 96.93$, $SEM = 2.04$; placebo: $M = 4.02$, $SEM = 0.39$) and after the Taylor

Aggression Paradigm (cortisol: $M = 89.80$, $SEM = 3.47$; placebo: $M = 4.11$, $SEM = 0.44$). Also, participants were not aware, whether they were in the cortisol or the placebo group, respectively.

Table 2 Characteristics of the subjects in the four groups: high provocation/cortisol ($n = 11$), high provocation/placebo ($n = 11$), low provocation/cortisol ($n = 13$) and low provocation/placebo ($n = 13$)

	high provocation/ $M (SD)$	high provocation/ $M (SD)$	low provocation/ cortisol $M (SD)$	low provocation/ placebo $M (SD)$	Statistical test value	p -Value
Number women/men	6/5	5/6	6/7	7/6	$\chi^2(3) = 0.37$.953
Age (years)	22.55 (2.38)	23.55 (3.96)	23.38 (2.02)	22.69 (2.29)	$F(3,44) < 1$.762
Time of experiment (h)	15:33 (02:04)	15:27 (02:29)	14:55 (02:37)	15:07 (02:45)	$F(3,44) < 1$.922
Wake-up time (h) ^a	07:17 (00:52)	07:07 (00:39)	07:15 (00:35)	07:02 (00:42)	$F(3,43) < 1$.828
Sleep duration (h) ^a	07:00 (00:49)	06:58 (01:11)	07:16 (00:56)	06:18 (00:44)	$F(3,43) < 1$.757
AUC _G (nmol/l) ^b	701.10 (240.70)	598.95 (232.55)	720.87 (261.48)	796.50 (221.39)	$F(3,44) = 1.36$.266
baseline cortisol (C1) ^c	0.75 (0.13)	0.67 (0.18)	0.66 (0.20)	0.63 (0.22)	$F(3,44) < 1$.516
cortisol at start of TAP (C2)	1.99 (.03)	0.51 (0.19)	1.97 (0.07)	0.61 (0.17)	$F(1,46) = 1324.44$.000
number placebo/cortisol ^e	5/6	8/3	9/4	11/2	$\chi^2(1) = 0.89$.344

^a $n = 47$

^b AUC_G = Area under the curve with respect to ground of the cortisol awakening response

^c log transformed values in nmol/l

^d Coparison between entire cortisol and placebo groups directly before the Taylor Aggression Paradigm (TAP)

^e Amount of subjects that thought they had gotten a placebo/cortisol tablet, comparison between cortisol and placebo groups

Figure 5 shows the descriptive values for all of the cells of the design, with the four graphs representing the aggressive responding in the high and the low provocation groups separately for males and females in the cortisol and placebo groups, respectively. Through the administration of cortisol, women became generally more aggressive (4b vs. 4d). The response pattern in the Taylor Aggression Paradigm of women receiving cortisol was very similar to the pattern seen in males under cortisol treatment (4c vs. 4d).

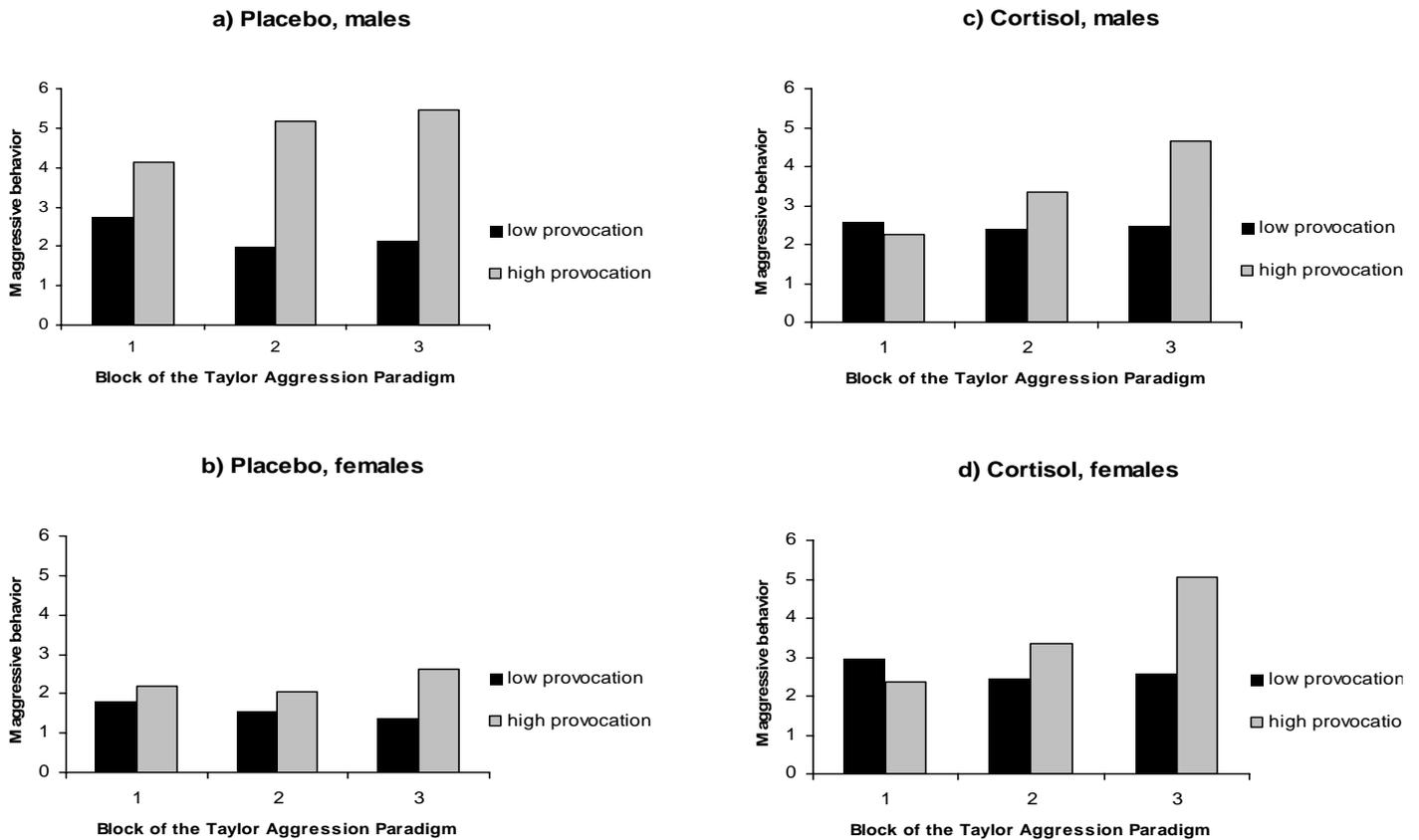


Figure 5 Mean aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the low and high provocation condition displayed for each gender/treatment condition (high provocation/cortisol/males ($n = 5$); high provocation/placebo/males ($n = 6$); low provocation/cortisol/males ($n = 7$); low provocation/placebo/males ($n = 6$); high provocation/cortisol/females ($n = 6$); high provocation/placebo/females ($n = 5$); low provocation/cortisol/females ($n = 6$); low provocation/placebo/females ($n = 7$)).

3.3.2 The effect of provocation in the Taylor Aggression Paradigm and cortisol administration on negative affect and angry feelings

For the item "angry feelings", the main effect of provocation group ($F(1,44) = 8.349, p = .006, \omega^2 = .13$), the main effect of measurement time ($F(1,44) = 19.928, p = .000, \omega^2 = .16$), as well as the interaction of provocation group and measurement time ($F(1,44) = 15.899, p = .000, \omega^2 = .13$) were significant. Post-hoc tests indicated that while participants in the low and high provocation group did not differ before the Taylor Aggression Paradigm in their feelings of anger, participants in the high provocation group had significantly more angry feelings after the Taylor Aggression Paradigm compared to before and compared to the low provocation group after the task. There were no main or interaction effects concerning treatment (all F 's < 1). For the negative affect scale, results were similar: Although the main

effect of measurement time was not significant ($F(1,44) < 1$), the main effect of provocation group ($F(1,44) = 5.952, p = .019, \omega^2 = .09$), as well as the interaction were significant ($F(1,44) = 15.390, p = .000, \omega^2 = .13$). Overall, the high provocation group showed more negative feelings than the low provocation group. The post-hoc test for the interaction indicated that this was only true after the Taylor Aggression Paradigm. There were no differences in negative affect prior to the task. Again, there were no effects involving treatment.

3.3.3 The effects of provocation, HPA axis activity, and gender on aggressive behavior

As expected, high provocation led generally to more aggressive behavior ($M = 3.55, SEM = 0.30$) than low provocation ($M = 2.26, SEM = 0.22$). The main effect of provocation ($F(1,32) = 12.300, p = .001, \omega^2 = .19$), the main effect of Block of the Taylor Aggression Paradigm ($F(2,64) = 9.736, p = .000, \omega^2 = .11$), as well as the interaction of the two ($F(2,64) = 21.562, p = .000, \omega^2 = .22$) were significant. The post-hoc test of the effect of Block of the Taylor Aggression Paradigm revealed that the participants were significantly more aggressive in Block 3 than in Block 1 or 2. Post-hoc tests of the interaction demonstrated that the high provocation group significantly increased their aggressive behavior over all blocks and this group showed significantly more aggressive behavior than the low provocation group in Block 2 and 3 of the Taylor Aggression Paradigm. The interaction is displayed in Figure 6.

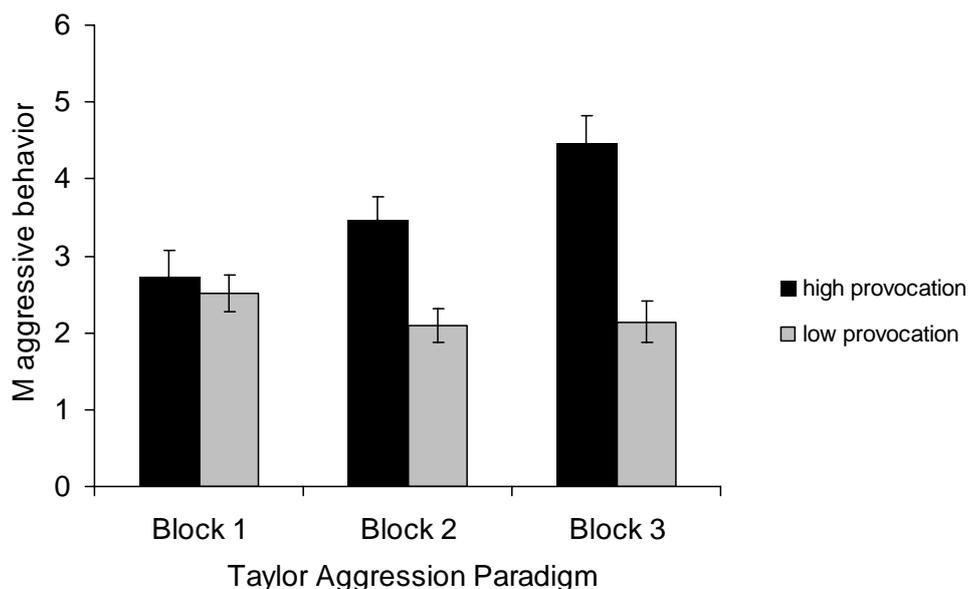


Figure 6 Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the low ($n = 26$) and high ($n = 22$) provocation group. Values are means \pm SEM.

The acute administration of cortisol had no overall effect on aggressive behavior ($F(1,32) < 1$), but interacted significantly with Block of the Taylor Aggression Paradigm ($F(2,64) = 4.728, p = .012, \omega^2 = .05$). Post-hoc tests indicated that the cortisol group was significantly more aggressive than the placebo group in Block 3 of the task, but not in Block 1 or 2. Furthermore, the cortisol group showed more aggressive behavior in Block 3 than in Block 1 or 2. The interaction is displayed in Figure 7. Furthermore, the cortisol treatment also significantly interacted with gender ($F(1,32) = 6.114, p = .019, \omega^2 = .10$; see Figure 8). The post-hoc tests indicated that males behaved more aggressively than females, but only in the placebo group. In the cortisol group, there was no difference between male and female aggression. The difference in aggression in females between the placebo and the cortisol group was significant. Although males tended to show less aggressive behavior under cortisol compared to the placebo condition, this decline was not significant and there were no significant higher order interactions including gender and treatment condition.

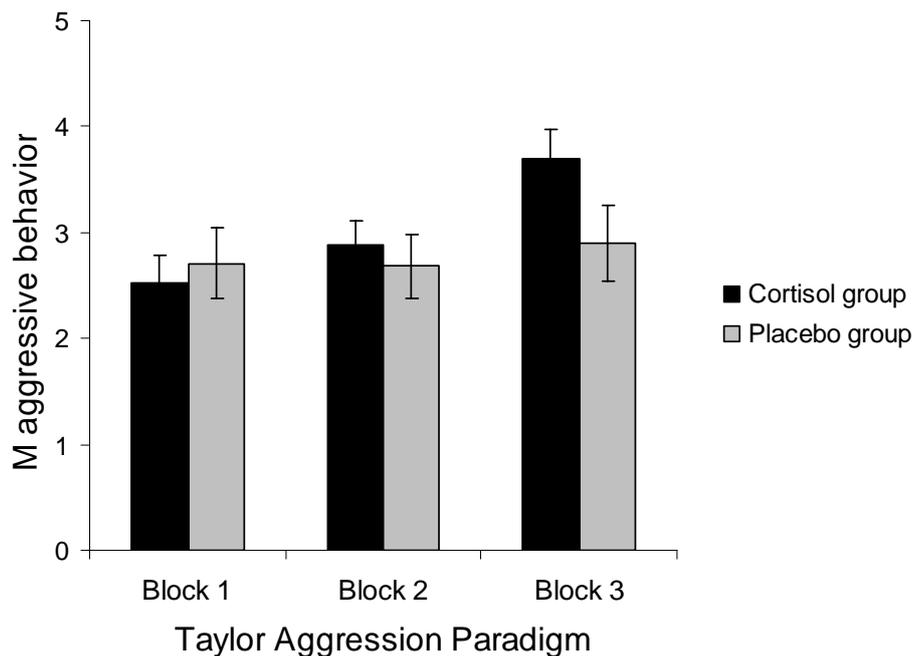


Figure 7 Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the cortisol ($n = 24$) and the placebo group ($n = 24$). Values are means \pm SEM.

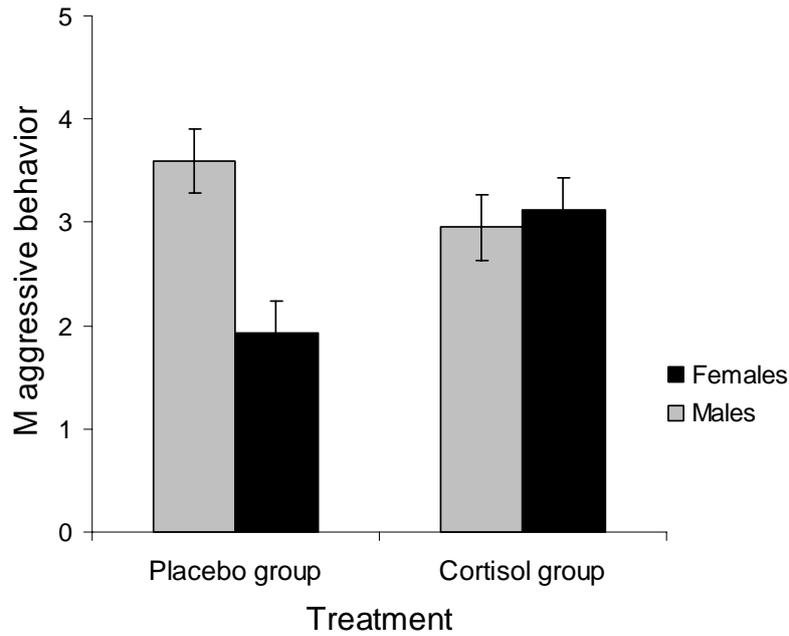


Figure 8 Mean aggressive behavior for males and females in the placebo and cortisol group, respectively (all $n = 12$). Values are means \pm SEM.

When looking at the placebo group, the average acute cortisol levels during the experiment tended to positively correlate with aggressive behavior, especially in block 2 of the task, but were not significant (mean aggressive behavior: $r = .219$; block 1: $r = .317$; block 2: $r = .309$; block 3: $r = .042$, all p 's $> .05$). However, the low and high provocation groups were mixed in this analysis. The correlations were much stronger and in some cases significant, if only the high provocation group was analyzed (mean aggressive behavior: $r = .599$, $p = .051$; block 1: $r = .576$, $p = .064$; block 2: $r = .778$, $p = .005$; block 3: $r = .198$, $p = .560$), whereas correlations for in the low provocation groups were close to zero and not significant ($.145 < r$'s $< .168$).

Within the analysis of variance, basal HPA axis activity had no general effect on aggression ($F(1,32) = 1.135$, $p = .295$), but it interacted significantly with gender and Block of the task ($F(1,64) = 6.694$, $p = .002$, $\omega^2 = .07$). A negative relationship between the cortisol awakening response and aggressive behavior was only apparent for women and got stronger over the course of the three blocks (Block 1: $r = -.168$; Block 2: $r = -.311$; Block 3: $r = -.381$). For men, the correlations were around zero in all three blocks. This interaction was further qualified by a four way interaction involving provocation group ($F(1,64) = 5.176$, $p = .008$, $\omega^2 = .05$). While men showed positive correlations between basal cortisol levels and aggression in the low provocation group, there was no discernable pattern for men in the high

provocation group. In women in both the low and high provocation group, the correlations were negative and again strongest in the third block of the task. Furthermore, as predicted, basal cortisol levels interacted significantly with cortisol group and Block of the Taylor Aggression Paradigm ($F(1,64) = 5.169, p = .008, \omega^2 = .05$), in that the negative correlation was only visible in the placebo, but not in the cortisol group. Again, the negative correlations got stronger over the three blocks, with a correlation of $r = -.409$ in the third block (Block 1: $r = -.087$; Block 2: $r = -.210$)².

3.4 Discussion

The aim of our study was to investigate the relationship of characteristics of HPA axis activity and experimentally induced aggressive behavior. Our findings clearly show that both an acute dose of hydrocortisone as well as basal HPA axis activity were important factors in explaining aggressive behavior. However, the effects depended on gender, as the relationship between the HPA axis and aggressive behavior were only visible in women, but not in men. In women, an acute dose of hydrocortisone lead to increased aggressive behavior and basal HPA axis activity was negatively correlated with aggressive behavior.

The induction of aggression with the Taylor Aggression Paradigm was successful. Participants in the high provocation group reacted significantly more aggressively in Block 2 and 3 of the task than the low provocation group. Furthermore, aggressive behavior significantly increased over the three blocks in the high provocation group, while there was no change in aggression in the low provocation group. 22% of the behavioral variance could be accounted for by the interaction of provocation and Block of the Taylor Aggression Paradigm. Moreover, participants in the high provocation group reported more negative feelings and particularly more feelings of anger after the task than the low provocation group, further validating the procedure. There were no effects of cortisol administration on negative affect and anger.

The acute dose of hydrocortisone showed no overall effect on aggressive behavior, but rather interacted with Block of the Taylor Aggression Paradigm and gender. Participants who were treated shortly before the task with a 20mg dose of hydrocortisone reacted more aggressively

² Since we found slightly positive associations between basal cortisol levels and wake-up time and AUC of the cortisol awakening response (see section 3.1), we wanted to rule out that effects of basal cortisol levels on aggressive behavior were caused by wake-up time. Therefore, we repeated the analysis with mean wake-up time instead of basal cortisol levels. All main and interaction effects concerning wake-up time were not significant (all F 's < 1). So aggressive behavior was truly related to cortisol levels and not to this confounding factor.

in Block 3 of the task than those participants treated with a placebo. Furthermore, the participants in the cortisol group became more aggressive over the course of the task with a significant increase from Block 1 and 2 to Block 3. In contrast to the hypothesis, the difference between the cortisol and the placebo group were apparent in the high as well as the low provocation group, i.e., the amount of provocation (low vs. high) did not moderate this relationship. Even in the condition where the participants received constantly low levels of provocation, aggression in Block 3 was higher for the cortisol compared to the placebo group. So, irrespective of how the participants got treated by their opponents, the participants with higher acute cortisol levels behaved more aggressively. When looking at the correlations of acute cortisol levels and aggressive behavior in the placebo group, moderate positive correlations were found. Correlations were especially strong and in most cases at least marginally significant when only the high provocation group was analyzed. This further underlines the importance of acute HPA axis activity in aggressive behavior. However, these results are only based on a small subsample and have to be interpreted with caution. Furthermore, the afternoon was chosen as time for the experiment to assure homogenous groups with respect to endogenous cortisol levels. This makes it difficult to find associations with other variables and might have contributed to the small correlations.

Recently, an integrative model of the neuronal, neurophysiological and psychological underpinnings of impulsive aggressive behavior was proposed (Strüber et al., 2008). Impulsive or reactive aggression is a form of aggression which is more uncontrolled and typically accompanied by anger or fear and by high levels of arousal (van Goozen et al., 2007) and may therefore be the type of aggression shown in the high provocation group (Bettencourt et al., 2006). Within the model of Strüber et al., aggression is thought to result from reduced impulse control. Normally, an active orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) as well as an inactive amygdala contribute to the inhibition of impulsive actions. But under certain situational aspects such as provocation, the inhibition through these systems is reduced, leading to more aggression. Cortisol might modulate these systems, lowering the inhibitory control of the OFC and ACC. This is supported by a functional magnetic resonance imaging (fMRI) study, where aggressive patients with a hyperreactivity of the HPA axis had a reduced activation of the OFC as well as a heightened activity of the amygdala when viewing social threat signals (Coccaro et al., 2007).

The effects of cortisol on frontal activity and a lowering of inhibitory control may be reflected by changes in information processing (Erickson et al., 2003), specifically the attention to, perception, and interpretation of social (threat-related) stimuli (Kruk et al., 2004). This may lead to heightened aggressive behavior in threatening (high provocation) as well as neutral or ambiguous situations (low provocation), because they are suddenly interpreted as hostile. Indeed, highly aggressive children have problems with the recognition of facial expressions (Fairchild et al., 2009) and interpret ambiguous signals as hostile (Dodge and Coie, 1987; Crick and Dodge, 1996; Kempes et al., 2005). Support for the hypothesis that this effect is mediated by cortisol comes from a recent study by Lopez-Duran and colleagues (2009), who found a positive association between HPA axis reactivity and reactive aggression as measured with a questionnaire. In addition to these correlative results, there is evidence that cortisol is causally involved in changing the attention to and processing of threat-related social stimuli (Putman et al., 2007; Roelofs et al., 2007). These results suggest that cortisol is necessary for information processing changes to occur that may precede aggressive behavior. To relate these considerations to our results, participants with elevated cortisol levels might have a changed information processing in that high and even low levels of provocation are interpreted as hostile and thus, reacted more aggressively.

Besides the interaction of treatment with Block of the Taylor Aggression Paradigm, treatment also interacted significantly with gender. The predicted effect of males being more aggressive than females was found in the placebo, but not in the cortisol group, where males and females showed equal levels of aggressive behavior (see Figure 5 & 5). Through the acute enhancement of cortisol levels, females showed more aggressive behavior than in the placebo condition resulting in a comparable intensity of aggressive behavior to that of males. The difference in the placebo group between males and females reflects the usual difference in aggression levels (Bettencourt and Miller, 1996). This gender difference may arise from differences in self-control mechanisms, with women having more self-control than men (Knight et al., 2002), although other factors, such as more efficient emotion-regulation may play a role, as well (Cappadocia et al., 2009). This difference between males and females is reflected in the above mentioned model of Strüber et al. by males having less inhibitory control over their impulsive actions and therefore higher levels of aggression in general, although this prediction could not be tested in our study. Since inhibition is already low in men, it is not further reduced by an acute activation of the HPA axis. But in women, high levels of HPA axis activity seem to impede normally strong inhibitory processes, leading to

less inhibition and to more aggressive behavior in females and therefore to equally high aggressive behavior in both sexes. This is supported by a meta-analysis on arousal and gender effects on aggression, where high levels of arousal lead to an abolishment of the gender difference in aggression usually observed in less arousing situations (Knight et al., 2002). With respect to gender, our results are not consistent with the studies by Verona and colleagues reported in the introduction, who mostly found an enhancement of aggressive behavior in males following a stressor, but not in females. There are several differences in the design of their and our studies. First, stress in those studies was induced with a physical stressor. The mechanism of such a stressor may be inherently different than that following a pharmacological manipulation of cortisol itself and therefore may have different neuronal and behavioral effects. Second, in the studies by Verona and colleagues, aggression was measured directly following or even during the stress induction, whereas we measured aggression when cortisol levels were probably already high for some time. We therefore speculate that the effects of cortisol might have a different time-response curve for males and females, with quicker effects for males that may already have passed before we began to measure aggression.

Basal HPA axis activity was also related to aggressive behavior, but it had distinct and additional effects from acute cortisol levels. Although there was no main effect of basal HPA axis activity and no interaction of provocation group and basal HPA axis activity, gender was an important moderator of the relationship of basal HPA axis activity and aggression. Only female participants exhibited a negative relationship and the correlations got stronger over the course of the task. Additionally, the negative correlations were found within the low and high provocation group. This negative relationship between basal cortisol levels and aggressive behavior fits well to previously reported results in females with clinical disorders. Pajer et al. found lower basal cortisol levels in girls with conduct disorder as compared to normal controls, especially for those girls with no other psychiatric problems (Pajer et al., 2001). Furthermore, lower cortisol to DHEA ratios were found in girls, and who had conduct disorder and at the same time exhibited increased aggressive behavior (Pajer et al., 2006). In the males, however, the correlations in all groups were close to zero and in some cases even positive. This is in contrast to many studies conducted with male child and adolescent clinical samples that mostly showed a negative relationship. Our sample is unique as it explores the relationship of basal HPA axis activity and aggression in healthy adults. Since it was shown that the effects of basal HPA axis activity on aggression are age-dependent (Alink et al.,

2008), it might explain why the relationship was not found in this sample. Also, the relationship might be more visible in clinical samples, as some studies with subclinical or community-based male samples also failed to find a negative association (Nickel et al., 2005; van Bokhoven et al., 2005; Fairchild et al., 2008; Marsman et al., 2008).

As predicted, basal HPA axis activity interacted with cortisol group and with Block of the Taylor Aggression Paradigm. Lower basal HPA axis activity was related to enhanced aggressive behavior, but this negative relationship was only apparent in the placebo group and was strongest in Block 3 of the task. So only when no acute activation of the HPA axis took place did basal HPA axis activity have an effect on aggressive behavior. This was what we expected, since Haller and colleagues (Haller et al., 2001) have shown in rats that while chronically low basal corticosterone levels caused abnormal aggressive behavior, an acute dose of corticosterone abolished this effect. We conclude that as the situation becomes demanding, i.e., apparent high activity of the HPA axis, the acute biological activity has a greater influence on behavior than more trait-like biological characteristics.

Our study has several strengths as it expands on other studies in the field. We used a validated procedure to induce and measure aggression under controlled laboratory conditions and acutely enhanced cortisol levels in a randomized sample. This made it possible to causally interpret the effect of an acute enhancement of cortisol levels on aggressive behavior and therefore extends on correlative evidence from previous studies. We assessed trait aspects of basal HPA axis activity with multiple measures across days. It could be shown, that the majority of variance in single measurements of baseline cortisol levels is caused by situational factors and that many measurements have to be taken in order to assess trait-like characteristics of the HPA axis (Hellhammer et al., 2007).

However, some limitations have to be addressed. First, we used a pharmacological agent to manipulate the HPA axis. This made it possible to look at specific effects of cortisol. However, cortisol only represents a part of the natural stress response (de Kloet et al., 2005), making it difficult to generalize the results to physical or psychological stressors. Second, we only included women who used oral contraceptives, so results can only be generalized to this specific population. Third, although the sample size altogether was adequate, we could only implement a between-subjects design due to the nature of the aggression provocation, leading to relatively small numbers of subjects within each cell. Therefore, baseline differences

between groups on various variables might have also contributed to the effects. The correlations within subgroups were also based on only a relatively small number of subjects and will have to be replicated by further studies. Fourth, the paradigm that was implemented in the study did not make it possible to compare different subtypes of aggression and their relation with HPA axis activity. This is relevant since studies have shown that the biological basis for each aggression subtype is different in humans (for a review, see Nelson and Trainor, 2007; Strüber et al., 2008). Future studies should therefore implement a paradigm which allows for the measurement of different aggression subtypes and to use questionnaires to validate the distinction. Fifth, we proposed in the discussion of gender differences that the time course of cortisol effects may be different for males and females. In order to test this hypothesis, it would be therefore good to vary the time between cortisol administration and provocation in future studies. And finally, these results were uncovered using a laboratory-induced provocation paradigm. Future studies should also be conducted in other settings, as well, since different processes may be at play in the naturally occurring manifestation of aggression.

We conclude that cortisol increased aggressive behavior in women, in both low and high provoking settings. This led to about the same levels seen in male participants, probably by impeding control processes that normally lead to a stronger regulation of such behavior in females. To our knowledge, this is the first evidence of a causal role of stress-related physiological activation in aggression in humans. Basal HPA axis activity seems to have an effect on aggressive behavior only in females and may be especially important in situations with no acute physiological activation. These results support the notion of a different biological basis of aggression in males and females, at least within healthy adults.

3.i Acknowledgments

This research was supported by the International Research Training Group "The Psychoneuroendocrinology of Stress" of the Deutsche Forschungsgemeinschaft (DFG, GRK1389/1, Project H). We want to thank the reviewers for their help in improving the manuscript.

3.ii References

- Aiken, L.S., West, S.G., 1991. Multiple regression: testing and interpreting interactions. Newbury, Sage.
- Alink, L.R., van Ijzendoorn, M.H., Bakermans-Kranenburg, M.J., Mesman, J., Juffer, F., Koot, H.M., 2008. Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev Psychobiol.* 50, 427-450.
- Anderson, C.A., Bushman, B.J., 1997. External Validity of "Trivial" Experiments: the Case of Laboratory Aggression. *Review of General Psychology.* 1, 19-41.
- Anderson, C.A., Bushman, B.J., 2002. Human aggression. *Annu Rev Psychol.* 53, 27-51.
- Berman, M., Gladue, B., Taylor, S., 1993. The effects of hormones, Type A behavior pattern, and provocation on aggression in men. *Motivation and Emotion.* 17, 125-138.
- Bernstein, S., Richardson, D., Hammock, G., 1987. Convergent and discriminant validity of the Taylor and Buss measures of physical aggression. *Aggressive Behavior.* 13, 15-24.
- Bettencourt, B.A., Miller, N., 1996. Gender differences in aggression as a function of provocation: a meta-analysis. *Psychol Bull.* 119, 422-447.
- Bettencourt, B.A., Talley, A., Benjamin, A.J., Valentine, J., 2006. Personality and aggressive behavior under provoking and neutral conditions: a meta-analytic review. *Psychol Bull.* 132, 751-777.
- Cappadocia, M.C., Desrocher, M., Pepler, D., Schroeder, J.H., 2009. Contextualizing the neurobiology of conduct disorder in an emotion dysregulation framework. *Clin Psychol Rev.* 29, 506-518.
- Coccaro, E.F., McCloskey, M.S., Fitzgerald, D.A., Phan, K.L., 2007. Amygdala and orbitofrontal reactivity to social threat in individuals with impulsive aggression. *Biol Psychiatry.* 62, 168-178.
- Cohen, J., 1988. Statistical power analysis for the behavioral sciences. Hillsdale, Erlbaum.

- Crick, N.R., Dodge, K.A., 1996. Social information-processing mechanisms in reactive and proactive aggression. *Child Dev.* 67, 993-1002.
- de Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci.* 6, 463-475.
- Dodge, K.A., Coie, J.D., 1987. Social-information-processing factors in reactive and proactive aggression in children's peer groups. *J Pers Soc Psychol.* 53, 1146-1158.
- Dressendörfer, R.A., Kirschbaum, C., Rohde, W., Stahl, F., Strasburger, C.J., 1992. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *J Steroid Biochem Mol Biol.* 43, 683-692.
- Erickson, K., Drevets, W., Schulkin, J., 2003. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. *Neurosci Biobehav Rev.* 27, 233-246.
- Fairchild, G., van Goozen, S.H., Stollery, S.J., Brown, J., Gardiner, J., Herbert, J., Goodyer, I.M., 2008. Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. *Biol Psychiatry.* 64, 599-606.
- Fairchild, G., Van Goozen, S.H., Calder, A.J., Stollery, S.J., Goodyer, I.M., 2009. Deficits in facial expression recognition in male adolescents with early-onset or adolescence-onset conduct disorder. *J Child Psychol Psychiatry.* 50, 627-636.
- Genazzani, A.R., Mannella, P., Simoncini, T., 2007. Drospirenone and its antialdosterone properties. *Climacteric.* 10 Suppl 1, 11-18.
- Gerra, G., Zaimovic, A., Raggi, M.A., Moi, G., Branchi, B., Moroni, M., Brambilla, F., 2007. Experimentally induced aggressiveness in heroin-dependent patients treated with buprenorphine: comparison of patients receiving methadone and healthy subjects. *Psychiatry Res.* 149, 201-213.
- Giancola, P.R., Zeichner, A., 1995. Construct validity of a competitive reaction-time aggression paradigm. *Aggressive Behavior.* 21, 199-204.
- Giancola, P.R., Parrott, D.J., 2008. Further evidence for the validity of the Taylor Aggression Paradigm. *Aggress Behav.* 34, 214-229.

- Granger, D.A., Blair, C., Willoughby, M., Kivlighan, K.T., Hibel, L.C., Fortunato, C.K., Wiegand, L.E., 2007. Individual differences in salivary cortisol and alpha-amylase in mothers and their infants: relation to tobacco smoke exposure. *Dev Psychobiol.* 49, 692-701.
- Haller, J., van de Schraaf, J., Kruk, M.R., 2001. Deviant forms of aggression in glucocorticoid hyporeactive rats: a model for 'pathological' aggression? *J Neuroendocrinol.* 13, 102-107.
- Haller, J., Halasz, J., Mikics, E., Kruk, M.R., 2004. Chronic glucocorticoid deficiency-induced abnormal aggression, autonomic hypoarousal, and social deficit in rats. *J Neuroendocrinol.* 16, 550-557.
- Haller, J., Kruk, M.R., 2006. Normal and abnormal aggression: human disorders and novel laboratory models. *Neurosci Biobehav Rev.* 30, 292-303.
- Hays, W.L., 1974. *Statistics*. London, Holt, Rinehart & Winston.
- Hellhammer, J., Fries, E., Schweisthal, O.W., Schlotz, W., Stone, A.A., Hagemann, D., 2007. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. *Psychoneuroendocrinology.* 32, 80-86.
- Huynh, H., Feldt, L.S., 1976. Estimation of the box correction for degrees of freedom from sample data in randomized block and splitsplot designs. *Journal of Educational Statistics.* 1, 69-82.
- Kempes, M., Matthys, W., de Vries, H., van Engeland, H., 2005. Reactive and proactive aggression in children--a review of theory, findings and the relevance for child and adolescent psychiatry. *Eur Child Adolesc Psychiatry.* 14, 11-19.
- Knight, G.P., Guthrie, I.K., Page, M.C., Fabes, R.A., 2002. Emotional arousal and gender differences in aggression: a meta-analysis. *Aggress Behav.* 28, 366-393.
- Krohne, H.W., Egloff, B., Kohlmann, C.-W., Tausch, A., 1996. [Investigations with a German version of the Positive and Negative Affect Schedule (PANAS)]. *Diagnostica.* 42, 139-156.

- Kruk, M.R., Halasz, J., Meelis, W., Haller, J., 2004. Fast positive feedback between the adrenocortical stress response and a brain mechanism involved in aggressive behavior. *Behav Neurosci.* 118, 1062-1070.
- Kudielka, B.M., Kirschbaum, C., 2003. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology.* 28, 35-47.
- Lopez-Duran, N.L., Olson, S.L., Hajal, N.J., Felt, B.T., Vazquez, D.M., 2009. Hypothalamic pituitary adrenal axis functioning in reactive and proactive aggression in children. *J Abnorm Child Psychol.* 37, 169-182.
- Marsman, R., Swinkels, S.H., Rosmalen, J.G., Oldehinkel, A.J., Ormel, J., Buitelaar, J.K., 2008. HPA-axis activity and externalizing behavior problems in early adolescents from the general population: the role of comorbidity and gender The TRAILS study. *Psychoneuroendocrinology.* 33, 789-798.
- Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. *Nat Rev Neurosci.* 8, 536-546.
- Nickel, C., Lahmann, C., Tritt, K., Loew, T.H., Rother, W.K., Nickel, M.K., 2005. Short communication: Stressed aggressive adolescents benefit from progressive muscle relaxation: a random, prospective, controlled trial. *Stress and Health.* 21, 169-175.
- Pajer, K., Gardner, W., Rubin, R.T., Perel, J., Neal, S., 2001. Decreased cortisol levels in adolescent girls with conduct disorder. *Arch Gen Psychiatry.* 58, 297-302.
- Pajer, K., Tabbah, R., Gardner, W., Rubin, R.T., Czambel, R.K., Wang, Y., 2006. Adrenal androgen and gonadal hormone levels in adolescent girls with conduct disorder. *Psychoneuroendocrinology.* 31, 1245-1256.
- Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology.* 28, 916-931.

- Putman, P., Hermans, E.J., van Honk, J., 2007. Exogenous cortisol shifts a motivated bias from fear to anger in spatial working memory for facial expressions. *Psychoneuroendocrinology*. 32, 14-21.
- Roelofs, K., Bakvis, P., Hermans, E.J., van Pelt, J., van Honk, J., 2007. The effects of social stress and cortisol responses on the preconscious selective attention to social threat. *Biol Psychol*. 75, 1-7.
- Salvador, A., Suay, F., Martinez-Sanchis, S., Simon, V.M., Brain, P.F., 1999. Correlating testosterone and fighting in male participants in judo contests. *Physiol Behav*. 68, 205-209.
- Schreiber, J.E., Shirtcliff, E., Van Hulle, C., Lemery-Chalfant, K., Klein, M.H., Kalin, N.H., Essex, M.J., Goldsmith, H.H., 2006. Environmental influences on family similarity in afternoon cortisol levels: twin and parent-offspring designs. *Psychoneuroendocrinology*. 31, 1131-1137.
- Strüber, D., Lück, M., Roth, G., 2008. Sex, aggression and impulse control: an integrative account. *Neurocase*. 14, 93-121.
- Taylor, S.P., 1967. Aggressive behavior and physiological arousal as a function of provocation and the tendency to inhibit aggression. *J Pers*. 35, 297-310.
- van Bokhoven, I., Van Goozen, S.H., van Engeland, H., Schaal, B., Arseneault, L., Seguin, J.R., Nagin, D.S., Vitaro, F., Tremblay, R.E., 2005. Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *J Neural Transm*. 112, 1083-1096.
- van Goozen, S.H., Fairchild, G., 2006. Neuroendocrine and neurotransmitter correlates in children with antisocial behavior. *Horm Behav*. 50, 647-654.
- van Goozen, S.H., Fairchild, G., Snoek, H., Harold, G.T., 2007. The evidence for a neurobiological model of childhood antisocial behavior. *Psychol Bull*. 133, 149-182.
- Verona, E., Joiner, T.E., Johnson, F., Bender, T.W., 2006a. Gender specific gene-environment interactions on laboratory-assessed aggression. *Biol Psychol*. 71, 33-41.
- Verona, E., Curtin, J.J., 2006b. Gender differences in the negative affective priming of aggressive behavior. *Emotion*. 6, 115-124.
-

Verona, E., Reed, A., 2nd, Curtin, J.J., Pole, M., 2007a. Gender differences in emotional and overt/covert aggressive responses to stress. *Aggress Behav.* 33, 261-271.

Verona, E., Kilmer, A., 2007b. Stress exposure and affective modulation of aggressive behavior in men and women. *J Abnorm Psychol.* 116, 410-421.

Wommack, J.C., Delville, Y., 2007. Cortisol controls the pubertal development of agonistic behavior in male golden hamsters via type II corticosteroid receptors. *Horm Behav.* 51, 306-312.

Wüst, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000b. The cortisol awakening response - normal values and confounds. *Noise Health.* 2, 79-88.

Chapter IV

Influence of aggression on information processing in the emotional Stroop task - an event-related potential study

(Bertsch, Böhnke, Kruk & Naumann, 2009)

First Author: Katja Bertsch.

Co-Authors: Menno R. Kruk & Ewald Naumann.

4.0 Abstract

Aggression is a common behavior which has frequently been explained as involving changes in higher level information processing patterns. Although researchers have started only recently to investigate information processing in healthy individuals while engaged in aggressive behavior, the impact of aggression on information processing beyond an aggressive encounter remains unclear. In an ERP study, we investigated the processing of facial expressions (happy, angry, fearful, and neutral) in an emotional Stroop task after experimentally provoking aggressive behavior in healthy participants. Compared to a non-provoked group, these individuals showed increased early (P2) and late (P3) positive amplitudes for all facial expressions. For the P2 amplitude, the effect of provocation was greatest for threat-related expressions. Beyond this, a bias for emotional expressions, i.e., slower reaction times to all emotional expressions, was found in provoked participants with a high level of trait anger. These results indicate significant effects of aggression on information processing, which last beyond the aggressive encounter even in healthy participants.

Keywords

Attentional bias, interference, threat, angry face, emotional expression, ERP

4.1 Introduction

Aggression is a common social behavior in both humans and animals. Not surprisingly, aggression and violence are among the leading causes of death worldwide (e.g., more than 1.6 million lives in 2000) and exert enormous economic costs (Krug et al., 2002). Neuroscientific research has mainly focused on pathologic aggression (e.g., Blair, 2004; Raine, 1989; Raine & Venables, 1988). However, aggression is also common in psychologically and neurologically healthy individuals. Aggression is necessary for human survival as it serves important purposes of allowing an individual to compete effectively for limited resources and to establish and maintain his/her position in society. The omnipresence of aggression and its impact on our everyday lives highlights the importance of finding an explanation of its causes and underlying mechanisms.

Information processing patterns at "higher levels" (e.g., scripts and schemata) have frequently been proposed as a possible explanation for aggressive behavior (e.g., Anderson & Bushman, 2002; Bushman & Anderson, 2001; Dodge & Crick, 199; Huesmann, 1988). Even so,

researchers have started to investigate the influence of aggression on basic information processing operations in healthy individuals only in the last decade. These studies show alterations in the cortical activity of healthy participants *while* they were engaged in a reactive aggression paradigm (Krämer et al., 2007; 2008; Lotze et al., 2007). For instance, participants with a high level of trait aggression displayed an enhanced early frontal negative event-related potential (ERP) in trials with high provocation while deciding about punishing an opponent (Krämer et al., 2008). A similar study with functional magnetic resonance imaging (fMRI) (Krämer et al., 2007) revealed that, in this decision phase, the activity in the rostral and dorsal parts of the anterior cingulate cortex and the anterior insula was greater in highly provocative than less provocative trials. Activity in these brain areas has been associated with emotional processing and this might reflect heightened emotional involvement of the participants under high provocation. Furthermore, an increase in activity in the medial prefrontal cortex (mPFC) has been reported during retaliation in a similar paradigm (Lotze et al., 2007). Enhanced activity in the dorsal mPFC might represent a stronger need for conflict management and response selection in the provoking situations. Increased activity in the ventral mPFC might indicate affective processes, such as compassion with the opponent. Hence, these findings show changes in the processing of information *during* an aggressive encounter in a laboratory setting.

From a therapeutic point of view, it may be even more interesting to investigate why aggressive behavior is often hard to stop and why it is easily transferred from one setting to another. Thus, the aim of the current study is to discover possible alterations in psychophysiological indicators of information processing *after* an aggressive encounter.

So far, this has only been addressed by a few behavioral studies, which have mainly focused on the influence of trait anger on reaction times for responding to threat-related stimuli. These studies have revealed that trait anger (Cohen et al., 1998; Eckhardt and Cohen, 1997; van Honk et al., 2001a; 2001b), previous self-reported aggressive experience (Smith and Waterman, 2004), and criminal convictions for violent offending (Smith and Waterman, 2003) predict an information processing bias for threat- or aggression-related material in various cognitive tasks. Thus, individuals with a high level of trait anger, who have experienced many incidences of aggression or violence seem to spend more attention on threat- or aggression-related information than less angry, aggressive, or violent individuals. This results in slower reactions to stimuli which are threat-related (emotional Stroop task) or

surrounded by threat-related stimuli (visual search task) compared to neutral stimuli. In addition, there is evidence that participants with a high level of trait anger, who are experimentally induced to experience anger, process task irrelevant anger-related material in an emotional Stroop task (Cohen et al., 1998) and a visual search task (Eckhardt and Cohen, 1997). Nevertheless, the underlying mechanisms of these changes in information processing associated with anger and/or aggression remain unclear. Research has only investigated differences in behavioral responses (i.e., reaction times) between individuals with high and low levels of trait anger or self-reported aggression. However, impaired reaction times are only an indirect measure of attention towards threat-related material or of an information processing bias.

Recent neuroimaging studies have proposed the involvement of a neural network consisting of the amygdala, the ventral anterior cingulate, and the ventral striatum in the processing of facial signals of aggression (Beaver et al., 2008; Passamonti et al., 2008). In particular, the ventral striatum and its associated dopaminergic system seem to play a specific role in the recognition of angry facial expressions. Selectively impaired recognition of angry expressions has been reported in patients with lesions in the ventral striatum (Calder et al., 2004) as well as after the administration of the dopamine antagonist sulpiride to healthy participants (Lawrence et al., 2002). Thus, biased responses for angry or threat-related material in participants with high state and/or trait anger or aggression might stem from an increased striatal activity.

In addition to functional neuroimaging, event-related potentials (ERP) studies might help to shed light at attention-related cortical processes related to anger and aggression. Because of their excellent temporal resolution, ERPs allow a finer, more sensitive and more direct examination of differences in the time course and cortical resources of information processing (Hillyard and Kutas, 1983). This is especially important, as numerous behavioral studies have reported no differences in the reaction times of healthy participants towards threat-related and neutral stimuli in cognitive tasks like the emotional Stroop task or the visual search task (for a meta-analytic review see Bar-Haim, et al., 2007). However, even in the absence of behavioral effects, significant differences could be found in the ERPs in some of the studies (Bar-Haim et al., 2005; Bernat et al., 2001; Carretié et al., 2001a; Thomas et al., 2007; Weinstein, 1995).

For instance, Thomas et al. (2007) reported greater parietal positivities to threat-related

compared to neutral words in an emotional Stroop task with healthy individuals. Although there were no differences in the reaction times, increased P2 (150 to 210 ms) and P3 (340 to 600 ms) amplitudes indicate an enhanced processing of threat-related compared to neutral words. Similar ERP responses have been found during the processing of emotional material when pictures of facial expressions were used. These studies also found rapid effects (< 250 ms post-stimulus), indicating a very early preferential processing or categorization of emotional - especially threat-related - facial expressions (Ashley et al., 2004; Bar-Haim et al., 2005; Bediou et al., 2009; Eimer and Holmes, 2002; Williams et al., 2006), followed by an alteration of later stages of ERP responses (Eimer and Holmes, 2002; Schupp et al., 2004; Williams et al., 2006). Alterations in the P2 and P3 amplitudes are thus sensitive indicators for early and later processing stages of emotional information. However, the levels of trait or state anger or aggression were not measured in these studies.

In summary, research indicates strong influences of anger and/or aggression on information processing. Trait anger and trait aggression are associated with reaction time biases towards threat- and aggression-related stimuli. Furthermore, healthy individuals show altered information processing *while* involved in aggressive behavior. However, the influence of experimentally induced aggression on information processing *beyond* an aggressive encounter and its underlying neural mechanisms has not been reported. Therefore, the present paper reports on an ERP study that investigated this research question.

Like previous studies, our present ERP study used the emotional Stroop task (Williams et al., 1996) to investigate information processing biases. This task requires the participant to identify the color of an emotional word, picture, or facial expression as fast as possible while ignoring its emotional content. An information processing bias (or interference) is inferred when the color naming takes longer with a threat-related stimulus than with a neutral stimulus. This has been frequently reported for clinically and subclinically anxious individuals (e.g., Bar-Haim et al., 2007), but also for individuals with high levels of trait anger (see above). Before performing an emotional Stroop task with happy, neutral, angry, and fearful facial expressions, the participants took part in a competitive reaction time task. This task was a modified Taylor Aggression Paradigm (TAP, Taylor, 1967), which was used to induce aggression in half of our group of healthy participants.

We expected differences in the reaction times of the provoked and non-provoked participants

in the emotional Stroop task. In particular, we hypothesized an information processing bias, that is longer reaction times for angry and fearful facial expressions in the provoked participants. Furthermore, we anticipated an increase in both early (P2) and late (P3) positive amplitudes in the provoked participants compared to the non-provoked participants for angry and fearful facial expressions.

4.2 Material and Methods

4.2.1 Participants

Twenty students of the University of Trier (10 female and 10 male, mean age = 23 years, $SE = 0.60$, range = 20-29 years) took part in the study. Exclusion criteria were left-handedness, color blindness, psychiatric disorders, regular medication (besides contraceptives), or any acute or chronic medical disease. The study was approved by the local ethics committee. Participation was compensated with €30 (approximately US\$40). The participants were randomly assigned to either an experimental (provoked participants) or a control (non-provoked participants) group, but sex was balanced across groups (five male and female participants in each group).

4.2.2 Materials

The Taylor Aggression Paradigm. Aggression was elicited and assessed with a modified version of the Taylor Aggression Paradigm (TAP, Taylor, 1967). The TAP has shown good construct, external, discriminant, and convergent validity (Anderson et al., 1999; Bernstein et al., 1987; Giancola and Chermack, 1998; Giancola and Zeichner, 1995).

The participants were led to believe that they were playing a competitive reaction time game with another participant who they met before the experiment started. The TAP consisted of 30 trials, which were divided into three blocks of ten trials. The participants' task was to react as fast as possible to a green square by pressing a key. They were told that whoever reacted slower would receive a blast of aversive noise. Prior to each trial, the participants had to set the volume and the duration of a noise for the opponent on two separate scales each ranging from 0 to 10. Corresponding to the 11 levels, the duration could be varied between 0 (level 0) and 5 seconds (level 10) in 0.5 second increments. The volume varied between 60 (level 1) and 105 dB (level 10) in 5 dB increments. The level 0 on the volume scale corresponded to 0 dB. After each trial, the participants received feedback about the outcome of the trial, i.e., whether they won or lost, as well as about the opponent's settings. In fact, there was no

opponent and the outcome of the trials was held constant for all participants - each of them won and lost half of the trials. The experimenter also set in advance the "opponent's" volume and duration settings according to the block and experimental condition of the participant. During the first block, all participants received short and gentle noises when they lost a trial (volume: $M = 62.5$ dB, range 0-70 dB; duration: $M = .075$ s, range 0-1.5 s). Participants of the non-provoked group received noises of the same volume and duration during the second and third block, as well. Participants of the provoked group were exposed to noises of intermediate volume and duration in the second block (volume: $M = 82.5$ dB, range 75-90 dB; duration: $M = 2.75$ s, range 2-3.5 s) and of high volume and duration in the third block (volume: $M = 99$ dB, range 90-105 dB; duration: $M = 4.4$ s, range 3.5-5 s) when they lost a trial. The volume and duration settings of the participants were recorded in each trial from 0 to 10. For each participant and each trial, an average of the volume and duration setting was computed, except for those trials in which one of the settings was 0. In that case, the total score was set to 0, since no noise would have been presented to the opponent and this trial would not have constituted an aggressive act. Finally, the 10 trials which belonged to one block of TAP were averaged for each participant. These values were then used as the dependent variable of aggressive behavior in the statistical analysis.

The emotional Stroop task. Stimuli were taken from Ekman and Matsumoto's Japanese and Caucasian Facial Expressions of Emotion (JACFEE) and Japanese and Caucasian Neutral Faces (JACNeuF) (Matsumoto and Ekman, 1988). We used pictures of four male and four female faces, displaying happy, angry, fearful, and neutral expressions. Duplications of each picture were colored in transparent red, blue, yellow, and green, resulting in 32 different stimuli. In total, the emotional Stroop task consisted of eight practice trials and 256 experimental trials. Each trial comprised the presentation of a colored facial expression, which was backwardly masked after 26.7 ms (2 frames at 75 Hz), since backward masking after 25 to 30 ms has been reported to produce large effects in regard to anger (e.g., Putman et al., 2004; van Honk et al., 2001a). The masks were individually constructed for each facial expression and represented a distorted version of the picture, keeping hue and saturation constant. The mask remained on the screen until the participant responded by orally naming the color of the picture. The participants were instructed to respond as fast as possible, whilst making as few errors as possible. The responses were recorded via microphone, and reaction times (i.e., voice onset times) were measured for each trial. The voice onset times were measured online with a microphone and serial voice response box (both provided by

Psychology Software Tools, Inc.). Prior to each facial presentation, a fixation cross appeared at the center of the screen for 1990 ms (the timing of a single trial is displayed in Figure 9).

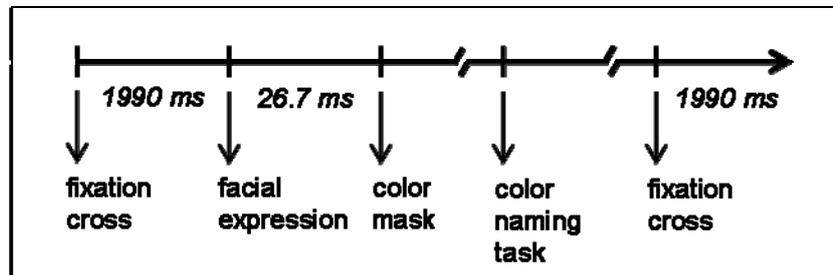


Figure 9 Time line for a single trial of the emotional Stroop task.

All stimuli were presented in the center of the screen on a black background. The image sizes were 5.55" x 5.20" and the vertical and horizontal visual angles were 0.28° and 0.26°, respectively. The stimuli were presented in a pseudorandomized fashion, which allowed a presentation of no more than three pictures of the same color or facial expression in a row. The task was divided into two random blocks of 128 pictures by a 2 min break.

Trait measures. Trait anger was measured prior to the experiment with the subscale anger of the Buss and Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1992; German version: Hewig et al., 2004). The BPAQ is a 29-item questionnaire, which consists of four subscales: anger, physical aggression, verbal aggression, and hostility. The four subscales have shown high internal and construct validity as well as high test-retest reliability (Buss and Perry, 1992; Harris, 1997). All items are scored using a 5-point Likert scale (1 = never or hardly ever applies to me, and 5 = very often applies to me). Two items on the scale are reverse scored. The BPAQ anger subscale is a good predictor for information processing biases in the emotional Stroop task and other cognitive tasks (Smith and Waterman, 2003, 2005).

4.2.3 Procedure

All participants were tested individually. We invited them to a preliminary interview, at which we checked for exclusion criteria and informed them about the aim of the study and the experimental procedure. They were told that the study would concern the investigation of the relationship between the steroid hormone cortisol, personality, and the perception of and reaction to visual stimuli. After a description of the experiment, the EEG and salivary cortisol measurement, written informed consent was obtained for all participants. Finally, the participants also received a battery of personality questionnaires to fill out at home as well as home sampling devices for salivary cortisol.

The experimental procedure was kept constant for all participants. On arrival, the participant was acquainted to another participant of the same sex, who was in fact a confederate of the experimenter. The participant and the confederate both handed over the filled out questionnaires and the salivary cortisol samples. The participants were then led to the EEG laboratory, where they were comfortably seated in a dimly lit sound-attenuated room one meter away from the 19" computer screen with a computer keyboard on a table in front of them. After the EEG electrodes were applied, the participants were left alone in the room for the remainder of the experiment and received all instructions via the computer screen. All participants first played the Taylor Aggression Paradigm, which lasted for about ten minutes, and then performed the emotional Stroop task for about 15 minutes. Before and after the TAP, as well as after the emotional Stroop task, the participants gave salivary cortisol samples, filled in a short mood questionnaire, and relaxed while the baseline resting EEG was measured during a 2 min period. A forth salivary cortisol sample was collected shortly before the participants left the laboratory. The results of these cortisol data are reported elsewhere (Böhnke et al., in review). Finally, the participants were debriefed about the true aim of the study as well as the TAP and the confederate. We thanked and compensated them for their participation.

Stimulus presentation and response logging were controlled using E-Prime software (Version 1.1, Psychology Software Tools, Inc.) and a serial voice response box and microphone. The experiment, including preparation and debriefing had a duration of 90 minutes.

4.2.4 EEG recording and quantification

The EEG was recorded from 32 electrode sites according to the 10-10 electrode reference system (Chatrian et al., 1988) including the mastoids with the Easy-Cap electrode system (Falk Minow Services). All sites were referenced to vertex (Cz). A bipolar horizontal electrooculogram (EOG) was recorded from the epicanthus of each eye, and a bipolar vertical EOG was recorded from supra- and infra-orbital positions of the left eye. The EEG and the EOG were recorded with Ag/AgCl electrodes. Prior to the electrode placement, the electrode sites on the participant's scalp and face were cleaned with alcohol and gently abraded. All impedances of the EEG electrodes were below 5 k Ω . EEG and EOG were amplified with a 32-channel SynAmps Model 5083 amplifier (input impedance: 10 M Ω ; Neuroscan, Inc.) in

AC mode. The pass-band was set to 0.05- to 40-Hz (-12 dB/octave rolloff); the signals were digitalized at 500 Hz and stored to hard disk for later analysis.

The EEG was re-referenced to linked mastoids. Artifacts due to eye movements were corrected via the algorithm developed by Gratton, Coles, and Donchin (1983). Trials with non-physiological artifacts were excluded from analysis via semiautomatic artifact rejection. EEG and EOG were epoched off-line into 1400-ms periods, starting 200 ms prior to stimulus onset and ending 1200 ms after stimulus onset. A baseline correction was performed using the first 200 ms as a reference. Separate averages were computed for each electrode, individual, and facial expression condition (happy, angry, fearful, and neutral).

Based on visual inspection of grand average ERPs, averaged across all participants and emotional facial expressions and a point-by-point inspection of effect sizes (Strelzyk et al., submitted) performed on all channels and time-frames, the following two stimulus-locked ERP components (peak amplitude relative to baseline) were identified and used for further analysis: P2 (the first major positive wave occurring 160-200 ms post-stimulus) and P3 (300-400 ms). The P2 and P3 waveforms had a centroparietal to parietal maximum. Therefore, we used the following nine central to parietal positions for further analyses: C3, Cz, C4, CP3, CPz, CP4, P3, Pz, and P4.

4.2.5 Statistical analyses

Aggressive behavior in the TAP. To check whether the induction of aggressive behavior in the provoked group was successful, we performed a 2×3 analysis of variance (ANOVA) including the factors provocation (provoked, non-provoked participants; between-subject) and TAP block (block 1, block 2, block 3; repeated measure).

Behavioral data in the emotional Stroop task. Outliers (± 2 SD) and trials with incorrect responses were individually rejected for each participant. We calculated the mean reaction time for each of the four emotional facial expression conditions. Bias scores were computed by subtracting the mean reaction time for neutral pictures from each of the three emotional categories (e.g., the individual mean response latencies for angry faces minus the individual mean response latencies for neutral faces) (e.g., van Honk et al., 2001a; Smith and Waterman, 2003; 2005). Note that positive bias scores are referred to as *interference* and negative scores as *facilitation*. To examine differences in reaction times towards facial expressions, we

submitted the mean correct responses to a 2×3 -mixed-design ANOVA examining the factors provocation (provoked, non-provoked participants; between-subject) and facial expression conditions (happy, angry, fearful; within-subject).

Electrophysiological data in the emotional Stroop task. For the ERP average amplitudes, we calculated separate $2 \times 4 \times 3 \times 3$ -mixed-design ANOVAs including the factors provocation (provoked participants, non-provoked participants; between-subject), facial expression conditions (happy, angry, fearful, neutral; repeated measure), caudality (central, centroparietal, parietal; repeated measures), and lateralization (left, middle, right; repeated measures) for each component (P2, P3).

Additional analyses. As trait anger has been previously found to be associated with an information processing bias (i.e., more interference) for threat-related stimuli (e.g., Eckhardt and Cohen, 1997; van Honk et al., 2001a; Smith and Waterman, 2003; 2005), we recalculated the statistical analyses for the behavioral and the electrophysiological data including the continuous between-subject factor of trait anger. For the *behavioral data*, we performed a 2×3 -mixed-design ANOVA including the factors provocation (provoked participants, non-provoked participants; between-subject), facial expression conditions (happy, angry, fearful; repeated measure), and trait anger (continuous between subject). Prior to this analysis, the mean trait anger scores were z-standardized (Aiken and West, 1991). For the *electrophysiological data*, separate $2 \times 4 \times 3 \times 3$ -mixed-design ANOVAs were calculated for the P2 and P3 components including the factors provocation (provoked participants, non-provoked participants; between-subject), facial expression conditions (happy, angry, fearful, neutral; repeated measure), caudality (central, centroparietal, parietal; repeated measure), lateralization (left, middle, right; repeated measure), and trait anger (continuous between subject; again, the z-standardized mean trait anger values were used for this analysis; Aiken and West, 1991).

For all ANOVAs, the degrees of freedom were Huynh-Feldt corrected if the assumption of sphericity was violated (Huynh and Feldt, 1976). We calculated Hays' ω^2 (Hays, 1974) as an effect size measure, with 1% considered a small effect, 5% considered a medium effect, and 14% considered a large effect (Cohen, 1988). A power analysis performed with GPOWER 2.0 (Buchner et al., 1996; Erdfelder et al., 1996) revealed a statistical power of $1-\beta \geq .90$ for

medium sized interaction effects of $\omega^2 = .05$ for the ERP data. According to Cohen (Cohen, 1962, 1988; 1992), values of $1-\beta \geq .80$ can be regarded as adequate statistical power for the interpretation of non-significant effects.

In case of significant effects, we used Dunn's Multiple Comparison Tests as well as Pearson product moment correlations as post hoc tests. All statistical analyses were conducted with SPSS for Windows (Version 14.0, SPSS Inc.).

4.3 Results

4.3.1 Aggressive behavior in the TAP

The experimental manipulation of aggressive behavior was successful. The provoked group showed generally more aggressive behavior ($M = 3.3$, $SE = 0.4$) than the non-provoked group ($M = 2.1$, $SE = 0.4$), $F(1,18) = 4.59$, $p = .046$, $\omega^2 = .15$. Aggressive behavior increased from the first to the third block of the TAP, $F(2,36) = 12.92$, $p = .001$, $\omega^2 = .28$. Post hoc tests showed significant differences between block 1 ($M = 2.1$, $SE = 0.3$) and block 3 ($M = 3.4$, $SE = 0.4$, $p < .010$) as well as between block 2 ($M = 2.6$, $SE = 0.3$) and block 3 ($M = 3.4$, $SE = 0.4$, $p < .010$) of the TAP. There was also a significant interaction between provocation and TAP block, $F(2,36) = 9.03$, $p = .003$, $\omega^2 = .21$. According to the post hoc test, aggressive behavior increased only in the provoked group ($p < .010$), but not in the non-provoked group. Moreover, the mean aggressive behavior of the groups differed in TAP block 2 ($p < .050$) and block 3 ($p < .010$), but not in block 1, where no provocation took place. Means and standard errors of each group and TAP block are presented in Figure 10.

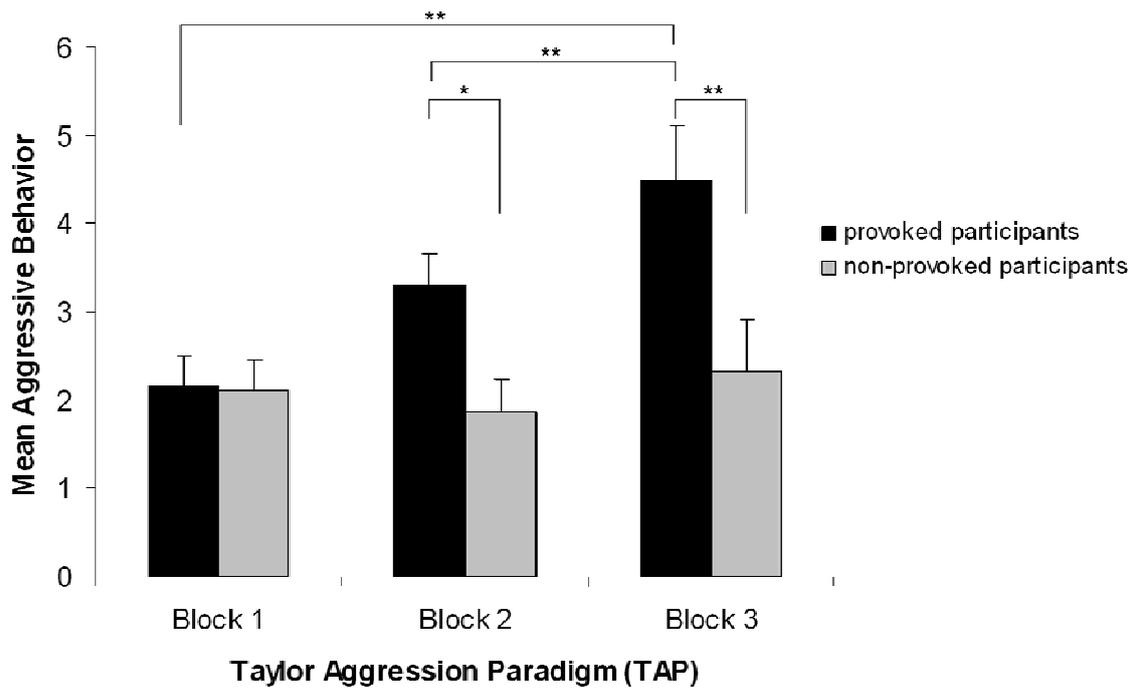


Figure 10 Mean aggressive behavior of the provoked and the non-provoked group in the three blocks of the Taylor Aggression Paradigm.

Note. Mean aggressive behavior represents the average of the loudness and duration setting. Each block consists of 10 trials (block 1: trial 1 to 10, block 2: trial 11 to 20, block 3: trial 21 to 30). The error bars represent ± 1 standard error.

4.3.2 Behavioral data in the emotional Stroop task

The error rate in this task was 2.19 % ($M = 5.6$, $SE = 0.8$) and the provoked and non-provoked participants did not differ in their error rates, $t < 1.00$, $p > .050$. The behavioral performance in the emotional Stroop task of the provoked and non-provoked participants is summarized in Table 3.

Analysis of the bias scores revealed a marginally significant main effect of provocation, $F(1,18) = 3.08$, $p = .096$, $\omega^2 = .09$, with provoked participants showing more interference ($M = 11.7$, $SE = 6.5$) for all emotional expressions (i.e., they were slower to name the color of emotional compared to neutral expressions) than the non-provoked participants ($M = -4.4$, $SE = 6.5$). Beyond this, no further significant effects were found (all F s < 1.0 , p s $> .050$).

Table 3 Reaction times and bias scores in the emotional Stroop task (means and standard errors).

	all participants ($N = 20$)		provoked participants ($N = 10$)		non-provoked participants ($N = 10$)	
	M	SE	M	SE	M	SE
RT neutral	527.9	12.8	526.8	23.7	529.1	11.5
RT happy	533.6	15.1	540.7	26.5	526.5	15.6
RT angry	530.8	15.6	537.4	27.6	524.1	15.8
RT fearful	530.4	14.5	537.5	26.6	523.3	13.1
bias happy	5.6	4.2	13.9	6.0	-2.6	6.0
bias angry	2.8	5.6	10.6	7.9	-4.9	7.9
bias fearful	2.5	5.4	10.7	7.6	-5.7	7.6

M = mean values (estimated marginals), SE = standard error. Note. Reaction time was measured in units of ms. Bias scores represent difference values (mean reaction times for emotional minus neutral expressions) in ms.

4.3.3 Electrophysiological data in the emotional Stroop task

Figure 11 shows grand average ERP responses to the facial expressions for the provoked and the non-provoked participants, averaged over the four facial expression conditions for all electrode positions. The general morphology of the waveform included a prominent, early negative peak at 135 ms (N1), followed by a positive wave at 180 ms (P2), a second negative wave at 250 ms (N2), and a final positive wave at 350 ms (P3). Descriptively large differences between the provoked and non-provoked participants can be observed in the time window of the P2 (160-220 ms) and P3 (300-400 ms) (see Figure 11).

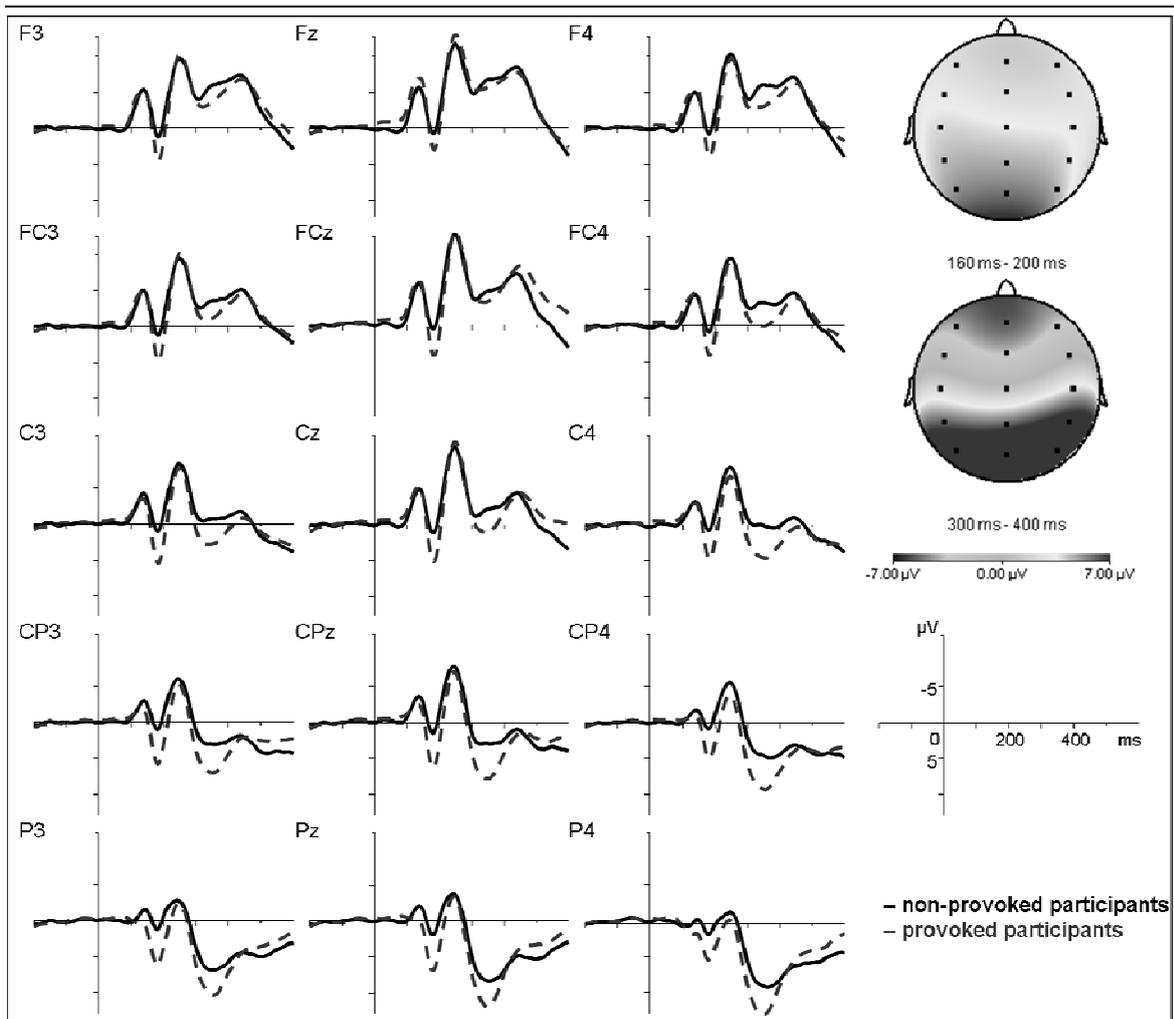


Figure 11 Grand average ERP waveforms for the provoked (--) and the non-provoked (–) group averaged over the four facial expressions (happy, neutral, fearful, and angry) and difference maps averaged over the four facial expressions (happy, neutral, fearful, and angry) for the time domains of the P2 (160-200 ms) and P3 (300-400 ms).

Note. In the difference maps, dark grey indicates a greater positivity in the provoked than in the non-provoked group and light grey refers to a greater positivity in the non-provoked compared to the provoked group.

P2 (160-200 ms). The P2 amplitude was greater in the provoked than in the non-provoked group, $F(1,18) = 5.77$, $p = .027$, $\omega^2 = .19$. This main effect of provocation was qualified by a significant interaction between provocation, caudality, and lateralization, $F(3,72) = 2.93$, $p = .031$, $\omega^2 = .04$. According to the post hoc tests, the group difference was greatest at P3 and Pz ($p < .010$). Moreover, there was a significant interaction between provocation and facial expression, $F(3,54) = 3.78$, $p = .016$, $\omega^2 = .09$ (see Figure 12). The post hoc tests indicated that the difference between the provoked and non-provoked participants was greatest for

fearful and angry expressions (both $ps < .001$), although significant group differences were also found for happy and neutral expressions (both $ps < .010$).

P3 (300-400 ms). We found a large main effect of provocation, $F(1,18) = 4.70$, $p = .004$, $\omega^2 = .16$, with a greater positivity in the provoked compared to the non-provoked participants. In addition, there was a significant interaction between facial expression and lateralization, $F(6,108) = 2.82$, $p = .014$, $\omega^2 = .04$. Post hoc tests showed that the *P3* amplitude was greater for happy than for neutral expressions at all electrode positions ($p < .05$), and greater for neutral than for angry expressions at right hemispheric electrode positions (C4, CP4, and P4; $p < .05$).

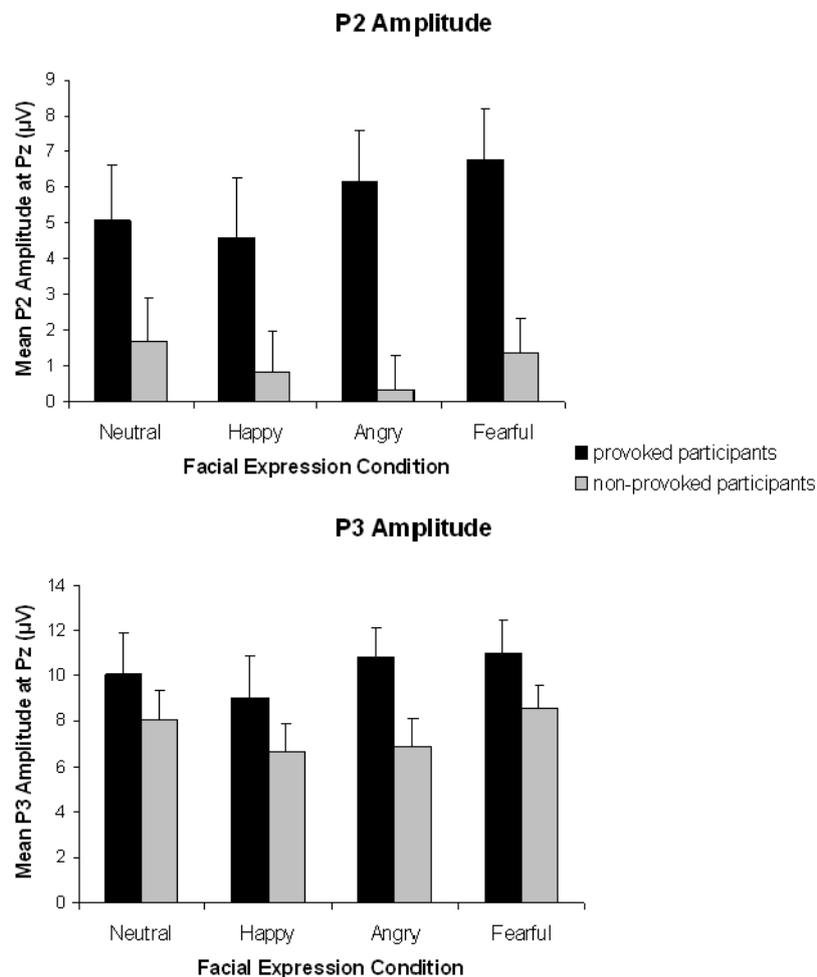


Figure 12 Mean P2 and P3 amplitudes (μV) for the provoked and non-provoked participants at Pz electrode site separately for the neutral, happy, angry, and fearful expressions.

4.3.4 Additional analyses

As in previous studies a greater information processing bias (i.e., more interference) was found in the emotional Stroop task for individuals with higher levels of trait anger, we recalculated the repeated measure ANOVAs and included the continuous between-subject factor of trait anger.

For the *behavioral data*, this analysis revealed a significant main effect of provocation, $F(1,18) = 7.10, p = .017, \omega^2 = .23$, a significant main effect of trait anger, $F(1,18) = 5.06, p = .039, \omega^2 = .17$, as well as a significant interaction between provocation and trait anger, $F(1,18) = 6.07, p = .025, \omega^2 = .20$. Again, the provoked participants showed more interference for all emotional expressions than the non-provoked participants. Bivariate correlations revealed a positive association between trait anger and the bias scores for all emotional expressions only in the provoked group, $.81 \leq r \leq .86, p \leq .005$. Thus, participants with a high level of trait anger who were experimentally provoked showed more interference to all emotional expressions (i.e., they were slower to name the color of emotional compared to neutral expressions) than participants with lower levels of trait anger and non-provoked participants.

For the *electrophysiological data*, the additional analyses did not reveal any further effects.

4.4 Discussion

Changes in information processing have been discussed in the context of aggression and higher level information-processing patterns (i.e., scripts or schemata), and have frequently been used to explain the occurrence of aggressive behavior (Anderson and Bushman, 2002; Dodge and Crick, 1990; Huesmann, 1988). There are also indications from recent ERP and fMRI studies that, even in healthy individuals, information processing is changed *while* they are involved in aggressive encounters (Krämer et al., 2008; Krämer et al., 2007; Lotze et al., 2007). Moreover, anger, self-reported aggression, and violent convictions have been associated with information processing biases for threat- and aggression-related material in several behavioral studies (Cohen et al., 1998; Eckhardt and Cohen, 1997; Smith and Waterman, 2003; 2004; van Honk et al., 2001a; 2001b). However, the influence of experimentally induced aggression on information processing and its underlying neural mechanisms has not been reported. Thus, in this ERP study, we measured reaction times as well as ERPs during the presentation of facial expressions in an emotional Stroop task

(Williams et al., 1996) after provoking aggressive behavior in half of our healthy participants with the Taylor Aggression Paradigm (Taylor, 1967).

The provocation of aggressive behavior was successful. On average, the participants in the provoked group set significantly louder and longer noises for their opponents when provoked (TAP block 2 and 3) compared to the non-provoked group and compared to TAP block 1, where no provocation took place. This experimental provocation of aggression led to a changed processing of facial expressions in both early and later stages of information processing, and on the behavioral level to more interference for emotional facial expressions.

4.4.1 Electrophysiological data in the emotional Stroop task

One aim of the study was to investigate differences in the ERPs directly after experimentally induced aggression as indicators for the processing of facial expressions in the emotional Stroop task. The principle advantage of ERPs is their excellent temporal resolution, which allows for the direct examination of differences in information processing and its time course (Hillyard and Kutas, 1983). The ERP results showed large differences between the provoked and non-provoked participants in two positive ERP components: the P2 and P3. This large main effect of provocation indicates that the experimental provocation had an impact on information processing in early as well as later stages of information processing, partly independent of the emotional content of the facial expressions.

First, the provoked participants showed an enhanced P2 amplitude compared to the non-provoked participants at posterior electrode positions. This very early component has been associated with bottom up or low level processing of information, such as stimulus classification and categorization (Crowley and Colrain, 2004). In this time window, we also found a significant interaction between provocation and facial expression condition, due to a greater positivity for threat-related (angry and fearful) expressions in the provoked participants. Similarly, Carretié et al. (2001b) reported a greater posterior P2 amplitude for negative compared to neutral and positive emotional pictures. This was interpreted in terms of a greater mobilization of attentional resources. Recently, Thomas et al. (2007) also found a greater P2 amplitude for threat-related compared to neutral words in an emotional Stroop task.

Schapkin et al (2000) understand the underlying processes of enhanced centro-parietal P2 amplitudes for emotional relative to neutral stimuli as an early global affective evaluation,

which appears to be critical for further approach or withdrawal behavior. According to studies by Calder and colleagues (Beaver et al., 2008; Calder et al., 2004; Lawrence et al., 2002; Passamonti et al., 2008), a neural network consisting of amygdala, anterior cingulate, and ventral striatum is involved in the processing of facial signals of aggression, i.e., angry facial expressions. One might speculate that the induction of aggression in the present study might have altered early global affective evaluation or categorization processes of all, and particularly threat-related facial expressions, potentially involving an altered striatal activity. However, this can only be resolved with studies using simultaneous measurements of EEG and fMRI, a now evolving technique (see e.g., Debener et al., 2006).

Second, we found a greater P3 amplitude in the provoked compared to the non-provoked participants. This component, which had a definite parietal localization, was independent of the emotional content of the facial expressions. An enhanced P3 with a centroparietal distribution has been previously found for less frequent, more salient and meaningful stimuli (Johnson, 1993; Naumann et al., 1992b; Picton, 1992). Unlike preceding ERP studies, we did not find a greater P3 amplitude for threat-related (Thomas et al., 2007) or emotional (Carretié et al., 2001a; Herbert et al., 2006; Naumann et al., 1992a) compared to neutral stimuli. Contrary to Thomas et al. (2007), we found significantly greater P3 amplitudes for neutral than for angry expressions, at least at right hemispheric electrode sites. However, Thomas et al. (2007) reported that P3 amplitude differences between threat and neutral words were considerably smaller when word meaning was not relevant for the task performance. It should be noted that there are several differences concerning the experimental design and material between the study of Thomas et al. (2007) and the present study (i.e., the use of angry and neutral words versus happy, angry, fearful, and neutral facial expression as well as a stimulus presentation time of 200 ms versus 26.7 ms). In particular, the different presentation times might at least partly account for the dissimilar results (see e.g., Kiss and Eimer, 2008). Moreover, none of those earlier studies included an experimental induction of aggression prior to the processing of emotional and neutral stimuli.

Recently, the P3 has been discussed with regard to the locus coeruleus norepinephrine (LC-NE) system (Nieuwenhuis et al., 2005). According to this theory, motivationally significant stimuli elicit a greater P3 amplitude due to a norepinephrine induced phasic enhancement of neural responsivity in the neocortex (especially the temporal-parietal junction). This enhancement is triggered by the outcome of task-relevant decision processes (e.g., stimulus

categorization). It is supposed to optimize information processing by modulating post-decision response processes. According to Nieuwenhuis et al. (2005), the LC-NE system might be a generator of the P3.

When provoked, all facial expressions are motivationally significant, because they might contain important and life-saving information about the opponent. Thus, provocation affects the processing of facial expressions at different levels. First, it alters early global affective evaluation processes. At this early stage of information processing, all facial expressions and particularly threat-related expressions are classified as motivationally significant (indicated by increased P2 amplitudes). Following this, an enhancement in the phasic LC-NE activity to *all* (motivationally significant) facial expressions results in an enhanced neural responsivity in the neocortex (indicated by increased P3 amplitudes). As mentioned above, such an interpretation has to be validated by joined fMRI and EEG measurements.

4.4.2 Behavioral data in the emotional Stroop task

Beyond the large group differences between provoked and non-provoked participants in the positive components of the ERPs, we also found behavioral effects related to the experimental induction of aggression. The provoked participants displayed more interference for all emotional (happy, angry, and fearful) facial expressions (i.e., they were slower to name the color of emotional compared to neutral facial expressions) than the non-provoked participants. This was especially the case when trait anger was included as a continuous between-subject factor. This additional analysis revealed positive correlations between trait anger and the bias scores for all emotional expressions within the provoked group, indicating slower reaction times in participants with high levels of trait anger after provocation.

This is partly in line with the results of previous behavioral studies, which found an information processing bias for threat-related material associated with anger and aggression (e.g., Eckhardt and Cohen, 1997; Cohen et al., 1998; van Honk et al., 2001a; 2001b; Smith and Waterman, 2003; 2004b). However, the induction of aggression in the present study resulted in a rather broad, less specific change of information processing and a processing bias (i.e., more interference) for all emotional facial expressions. In contrast, the information processing bias of participants with high levels of trait anger, self-reported experiences of aggression, or violent incidences, which has been reported by other studies, was specific for threat- or aggression-related material. So far, it remains unclear why induced aggression

should lead to a broader change in information processing. One could speculate that all emotional facial expressions gain relevance after being involved in an aggressive encounter. Facial expressions inform us more rapidly than language about the state of mind of other individuals and are, thus, biologically and socially salient stimuli in human nonverbal communication (Le Doux, 1998). Even a laughing face might be provoking in such a situation, as this person might be laughing at you. Moreover, the immediate and reliable awareness about potential friends and enemies might be more important and even lifesaving in the context of an acute aggressive encounter. This is supported by the ERP data of the present study. An increase in the P2 and P3 amplitudes in the provoked participants indicate a greater relevance and salience of all facial stimuli after an aggressive encounter independent of the individual's level of trait anger. In other words, the induction of aggression seems to produce a general gating effect of the neural response at the level of both early and later ERP components. Only in participants with high levels of trait anger, the provocation also resulted in behavioral differences, i.e. more interference for emotional expressions.

4.4.3 Limitations

Before strong conclusions can be drawn, two limitations of the present study should be noted. First, we did not include a control condition with non-facial stimuli in the present study. Hence, it remains unclear whether the changes in information processing due to aggression are specific for facial expressions or are more general, going beyond (or not depending upon) facial expressions.

Second, like previous studies using the emotional Stroop task (e.g., Putman et al., 2004, Smith and Waterman, 2003; 2004, 2005; van Honk et al., 2001a; 2001b), we requested the participants to orally name the color of the presented stimuli. This might have introduced artifacts in the EEG. However, it should be noted, that the mean response latencies (around 530 ms) did not overlap with the time domains of investigated components (160-200 ms and 300-400 ms).

Third, we only found a marginally significant and emotion-unspecific effect of aggression in the behavioral data. Greater behavioral effects were found, when trait anger was included as a continuous between-subject factor. However, the study was not designed to investigate interaction effects of trait anger. As the statistical power for the behavioral data is not adequate to interpret non-significant effects, these behavioral results need to be replicated in a

larger sample. Nevertheless, for the statistical analyses of the ERP data the statistical power was sufficient to interpret non-significant interaction effects (see Method and Material section). To overcome these limitations, a second study with more participants, which also includes a non-facial control condition, is in preparation.

4.4.4 Conclusion

In summary, this study showed that experimentally induced aggression has a strong impact on early as well as later stages of information processing. The ERPs revealed large differences between provoked and non-provoked participants during the processing of facial expressions in an emotional Stroop task, largely independent of the emotional content of the facial expressions and the individual level of trait anger. Moreover, aggression led to slower reaction times and therefore an information processing bias for emotional facial expressions, especially in participants with a high level of trait anger. Together with the findings from previous studies, our results demonstrate pronounced effects of aggression on information processing during and after an aggressive encounter. It is intriguing that even a mild provocation in a laboratory setting affects several stages of information processing and results in behavioral differences even up to 15 min after the aggressive encounter took place. This suggests profound effects from real-life conflicts and aggressive encounters on information processing and consequent behavior.

4.i Author Note

The authors are grateful to Renate Freudenreich, Helmut Peifer, Johann Kim, and Patrick Britz for their help with data acquisition and programming as well as their technical support. We also thank Terry Blumenthal, Florian Strelzyk, and Stefan Telega for helpful comments on an earlier version of this manuscript. The authors are members of the International Research Training Group "Psychoneuroendocrinology of Stress: From Molecules and Genes to Affect and Cognition" funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), grant GRK 1389/1.

Address reprint requests to: Katja Bertsch, FB I–Psychologie, Universität Trier, Universitätsring 15, 54286 Trier, Germany. Email: bert1301@uni-trier.de

4.ii References

- Aiken, L. S. and West, S. G. (1991). Multiple regression: testing and interpreting interactions. (Newbury: Sage).
- Anderson, C. A. and Bushman, B. J. (2002). Human aggression. *Annual Review of Psychology* 53, 27-51.
- Anderson, C. A., Lindsay, J. J. and Bushman, B. J. (1999). Research in the psychological laboratory: truth or triviality? *Current Directions in Psychological Science* 8, 3-9.
- Ashley, V., Vuilleumier, P. and Swick, D. (2004). Time course and specificity of event-related potentials to emotional expressions. *Neuroreport* 15, 211-216.
- Bar-Haim, Y., Lamy, D. and Glickman, S. (2005). Attentional bias in anxiety: a behavioral and ERP study. *Brain and Cognition* 59, 11-22.
- Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J. and van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin* 133, 1-24.
- Bar-Heim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J. and van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin* 133, 1-24.
- Beaver, J. D., Lawrence, A. D., Passamonti, L. and Calder, A. J. (2008). Appetitive motivation predicts the neural response to facial signals of aggression. *The Journal of Neuroscience* 28, 2719-2725.
- Bediou, B., Eimer, M., d'Aato, T., Hauk, O. and Calder, A. J. (2009). In the eye of the beholder: individual differences in reward-drive modulate early frontocentral ERP to angry faces. *Neuropsychologia* 47, 825-834.
- Bernat, E., Bunce, S. and Shevrin, H. (2001). Event-related potentials differentiate positive and negative mood adjectives during both supraliminal and subliminal visual processing. *International Journal of Psychophysiology* 42, 11-34.

- Bernstein, S., Richardson, D. and Hammock, G. (1987). Convergent and discriminant validity of the Taylor and Buss measures of physical aggression. *Aggressive Behavior* 13, 15-24.
- Blair, R. J. R. (2004). The roles of orbital frontal cortex in the modulation of antisocial behavior. *Brain and Cognition* 55, 198-208.
- Böhnke, R., Bertsch, K., Kruk, M. R. and Naumann, E. (in review). The influence of HPA-axis activity on aggression.
- Buchner, A., Erdfelder, E. and Faul, F. (1996). Teststärkenanalysen [Power analyses]. In *Handbuch Quantitative Methoden [Handbook quantitative methods]*, of Erdfelder, E. et al. (Eds.) (Weinheim: Psychologie Verlags Union), pp. 123-136.
- Bushman, B. J. and Anderson, C. A. (2001). Is it time to pull the plug on the hostile versus instrumental aggression dichotomy? *Psychological Review* 108, 273-279.
- Buss, A. H. and Perry, M. (1992). The aggression questionnaire. *Journal of Personality and Social Psychology* 63, 452-459.
- Calder, A. J., Keane, J., Lawrence, A. D. and Manes, F. (2004). Impaired recognition of anger following damage to the ventral striatum. *Brain* 127, 1958-1969.
- Carretié, L., Martín-Loeches, M., Hinojosa, J. A. and Mercado, F. (2001a). Emotion and attention interaction studies through event-related potentials. *Journal of Cognitive Neuroscience* 13, 1109-1128.
- Carretié, L., Mercado, F., Tapia, M. and Hinojosa, J. (2001b). Emotion, attention, and the 'negativity bias', studied through event-related potentials. *International Journal of Psychophysiology* 41, 75-85.
- Chatrian, G. E., Lettich, E. and Nelson, P. L. (1988). Modified nomenclature of the "10%" electrode system. *Journal of Clinical Neurophysiology* 5, 183-186.
- Cohen, D. J., Eckhardt, C. I. and Schagat, K. D. (1998). Attention allocation and habituation to anger-related stimuli during a visual search task. *Aggressive Behavior* 24, 399-409.
- Cohen, J. (1962). The statistical power of abnormal-social psychological research: a review. *Journal of Abnormal and Social Psychology* 65, 145-153.
-

- Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. (Hilldale, New York: Erlbaum).
- Cohen, J. (1992). A Power Primer. *Psychological Bulletin* 112, 155-159.
- Crowley, K. E. and Colrain, I. M. (2004). A review of the evidence for P2 being an independent component process: age, sleep and modality. *Clinical Neurophysiology* 115, 732-744.
- Debener, S., Ullsperger, M., Siegel, M. and Engel, A. K. (2006). Single-trial EEG-fMRI reveals the dynamics of cognitive function. *Trends in Cognitive Sciences* 10, 585-563.
- Dodge, K. A. and Crick, N. R. (1990). Social information-processing bases of aggressive behavior in children. *Personality and Social Psychology Bulletin* 16, 8-22.
- Eckhardt, C. I. and Cohen, D. J. (1997). Attention to anger-relevant and irrelevant stimuli following naturalistic insult. *Personality and Individual Differences* 23, 619-629.
- Eimer, M. and Holmes, A. (2002). An ERP study on the time course of emotional face processing. *Neuroreport* 13, 427-431.
- Erdfelder, E., Faul, F. and Buchner, A. (1996). GPOWER: A general power analysis program. *Behavior Research Methods, Instruments, & Computers* 28, 1-11.
- Giancola, P. R. and Chermack, S. T. (1998). Construct validity of laboratory aggression paradigms: a response to Tedeschi and Quigley (1996). *Aggression and Violent Behavior* 3, 237-253.
- Giancola, P. R. and Zeichner, A. (1995). Construct validity of a competitive reaction-time aggression paradigm. *Aggressive Behavior* 21, 199-204.
- Gratton, G., Coles, M. G. and Donchin, E. (1983). A new method for off-line removal of ocular artifacts. *Electroencephalography and Clinical Neurophysiology* 55, 468-484.
- Harris, J. A. (1997). A further evaluation of the aggression questionnaire: issues of validity and reliability. *Behavior Research and Therapy* 35, 1047-1053.
- Hays, W. L. (1974). *Statistics for the social sciences*. (London: Holt, Rinehart & Winston).

- Herbert, C., Kissler, J., Junghöfer, M., Peyk, P. and Rockstroh, B. (2006). Processing of emotional adjectives: evidence from startle EMG and ERPs. *Psychophysiology* 43, 197-206.
- Hewig, J., Hagemann, D., Seifert, J., Naumann, E. and Bartussek, D. (2004). On selective relation of frontal cortical asymmetry and anger-out versus anger-control. *Journal of Personality and Social Psychology* 87, 926-939.
- Hillyard, S. A. and Kutas, M. (1983). Electrophysiology of cognitive processing. *Annual Review of Psychology* 34, 33-61.
- Huesmann, L. R. (1988). An information processing model for the development of aggression. *Aggressive Behavior* 14, 13-24.
- Huynh, H. and Feldt, L. S. (1976). Estimation of the box correction for degrees of freedom from sample data in randomized block and split-plot designs. *Journal of Educational Statistics* 1, 69-82.
- Johnson, R. J. (1993). On the neural generators of the P300 component of the event-related potential. *Psychophysiology* 30, 90-97.
- Kiss, M. and Eimer, M. (2008). ERPs reveal subliminal processing of fearful faces. *Psychophysiology* 45, 318-326.
- Krämer, U. M., Büttner, S., Roth, G. and Münte, T. F. (2008). Trait aggressiveness modulates neurophysiological correlates of laboratory-induced reactive aggression in humans. *Journal of Cognitive Neuroscience* 20, 1464-1477.
- Krämer, U. M., Jansma, H., Tempelmann, C. and Münte, T. F. (2007). Tit-for-tat: the neural basis of reactive aggression. *NeuroImage* 38, 203-211.
- Krug, E. G., Dahlberg, L. L., Mercy, J. A., Zwi, A. B. and Lozano, R. (Eds.), 2002. Word report on violence and health. World Health Organization, Geneva.
- Lawrence, A. D., Calder, A. J., McGowan, S. W. and Grasby, P. M. (2002). Selective disruption of the recognition of facial expressions of anger. *Neuroreport* 13, 881-884.
- Le Doux, J. (1998). The emotional brain. (London: Weidenfeld & Nicolson).

- Lotze, M., Veit, R., Anders, S. and Birbaumer, N. (2007). Evidence for a different role of the ventral and dorsal medial prefrontal cortex for social reactive aggression: an interactive fMRI study. *NeuroImage* 34, 470-478.
- Matsumoto, D. and Ekman, P. (1988). Japanese and caucasian facial expressions of emotion and neutral faces (JACFEE and JACNeuF) [Slides]. (San Francisco, CA: Intercultural and Emotion Research Laboratory, Department of Psychology, San Francisco State University).
- Naumann, E., Bartussek, D., Diedrich, O. and Laufer, M. E. (1992a). Assessing cognitive and affective information processing functions of the brain by means of the late positive complex of the event-related potential. *Journal of Psychophysiology* 6, 285-298.
- Naumann, E., Huber, C., Maier, S., Plihal, W., Wustmans, A., Diedrich, O. and Bartussek, D. (1992b). The scalp topography of P300 in the visual and auditory modalities: a comparison of three normalization methods and the control of statistical type II error. *Electroencephalography and Clinical Neurophysiology* 83, 254-264.
- Nieuwenhuis, S., Slagter, H. A., von Geusau, N. J., Heslenfeld, D. J. and Holroyd, C. B. (2005). Knowing good from bad: differential activation of human cortical areas by positive and negative outcomes. *European Journal of Neuroscience* 21, 3161-3168.
- Passamonti, L., Rowe, J. B., Ewbank, M., Hampshire, A., Keane, J. and Calder, A. J. (2008). Connectivity from the ventral anterior cingulate to the amygdala is modulated by appetitive motivation in response to facial signals of aggression. *NeuroImage* 43, 562-570.
- Picton, T. W. (1992). The P300 wave of the human event-related potential. *Journal of Clinical Neurophysiology*, 456-479.
- Putman, P., Hermans, E. and van Honk, J. (2004). Emotional Stroop performance for masked angry faces: it's BAS, not BIS. *Emotion* 4, 305-311.
- Raine, A. (1989). Evoked potentials and psychopathy. *International Journal of Psychophysiology* 8, 1-16.
- Raine, A. and Venables, P. H. (1988). Enhanced P3 evoked potential and longer P3 recovery times in psychopaths. *Psychophysiology* 25, 30-38.

- Schapkin, S. A., Gusev, A. N. and Kuhl, J. (2000). Categorization of unilaterally presented emotional words: an ERP analysis. *Acta Neurobiologiae Experimentalis* 60, 17-28.
- Schupp, H. T., Öhman, A., Junghöfer, M., Weike, A. I., Stockburger, J. and Hamm, A. O. (2004). The facilitated processing of threatening faces: an ERP analysis. *Emotion* 4, 189-200.
- Smith, P. and Waterman, M. (2003). Processing bias for aggression words in forensic and nonforensic samples. *Cognition and Emotion* 17, 681-701.
- Smith, P. and Waterman, M. (2004). Role of experience in processing bias for aggressive words in forensic and non-forensic populations. *Aggressive Behavior* 30, 105-122.
- Smith, P. and Waterman, M. (2005). Sex differences in processing aggression words using the emotional Stroop task. *Aggressive Behavior* 31, 271-282.
- Strelzyk, F., Britz, P. and Naumann, E. (submitted). Advantages of effect-size measures for the data exploration of event-related potentials.
- Taylor, S. P. (1967). Aggressive behavior and physiological arousal as a function of provocation and the tendency to inhibit aggression. *Journal of Personality* 35, 297-310.
- Thomas, S. J., Johnstone, S. J. and Gonsalvez, C. J. (2007). Event-related potentials during an emotional stroop task. *International Journal of Psychophysiology* 63, 221-231.
- van Honk, J., Tuiten, A., De Haan, E., van den Hout, M. and Stam, H. (2001a). Attentional bias for angry faces: relationships to trait anger and anxiety. *Cognition and Emotion* 15, 279-297.
- van Honk, J., Tuiten, A., van den Hout, M., Putman, P., de Haan, E. and Stam, H. (2001b). Selective attention to unmasked and masked threatening words: relationships to trait anger and anxiety. *Personality and Individual Differences* 30, 711-720.
- Weinstein, A. M. (1995). Visual ERPs evidence for enhanced processing of threatening information in anxious university students. *Biological Psychiatry* 37, 847-858.
- Williams, J. M., Mathews, A. and MacLeod, C. M. (1996). The emotional Stroop task and psychopathology. *Psychological Bulletin* 120, 3-24.

Williams, L. M., Palmer, D., Liddell, B. J., Song, L. and Gordon, E. (2006). The 'when' and 'where' of perceiving signals of threat versus non-threat. *NeuroImage* 31, 458-467.

General References

- Alink, L. R., van Ijzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F. & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev Psychobiol*, 50(5), 427-450.
- Anderson, C. A. & Bushman, B. J. (2002). Human aggression. *Annu Rev Psychol*, 53, 27-51.
- APA. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision ed.). Washington, DC: American Psychiatric Association.
- Baron, R. A. & Richardson, D. R. (1994). *Human Aggression*. New York: Plenum Press.
- Bremner, J. D., Vythilingam, M., Vermetten, E., Adil, J., Khan, S., Nazeer, A., et al. (2003). Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse. *Psychoneuroendocrinology*, 28(6), 733-750.
- Cannon, W. (1932). *The wisdom of the body*. New York: W W Norton & Co.
- Chrousos, G. P. & Gold, P. W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *Jama*, 267(9), 1244-1252.
- Coccaro, E. F., McCloskey, M. S., Fitzgerald, D. A. & Phan, K. L. (2007). Amygdala and Orbitofrontal Reactivity to Social Threat in Individuals with Impulsive Aggression. *Biol Psychiatry*.
- Crick, N. R. & Dodge, K. A. (1994). A review and reformulation of social information-processing mechanisms in children's social adjustment. *Psychological Bulletin*, 115, 74-101.
- Crick, N. R. & Dodge, K. A. (1996). Social information-processing mechanisms in reactive and proactive aggression. *Child Dev*, 67(3), 993-1002.
- de Kloet, E. R., Joels, M. & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*, 6(6), 463-475.

-
- Elzinga, B. M., Schmahl, C. G., Vermetten, E., van Dyck, R. & Bremner, J. D. (2003). Higher cortisol levels following exposure to traumatic reminders in abuse-related PTSD. *Neuropsychopharmacology*, 28(9), 1656-1665.
- Eronen, M., Angermeyer, M. C. & Schulze, B. (1998). The psychiatric epidemiology of violent behaviour. *Soc Psychiatry Psychiatr Epidemiol*, 33 Suppl 1, S13-23.
- Fairchild, G., Van Goozen, S. H., Calder, A. J., Stollery, S. J. & Goodyer, I. M. (2009). Deficits in facial expression recognition in male adolescents with early-onset or adolescence-onset conduct disorder. *J Child Psychol Psychiatry*, 50(5), 627-636.
- Fairchild, G., van Goozen, S. H., Stollery, S. J., Brown, J., Gardiner, J., Herbert, J., et al. (2008). Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. *Biol Psychiatry*, 64(7), 599-606.
- Fava, M. (1998). Depression with anger attacks. *J Clin Psychiatry*, 59 Suppl 18, 18-22.
- Fulford, A. J. & Harbuz, M. S. (2005). An introduction to the HPA axis. In T. Steckler, N. H. Kalin & J. M. H. M. Reul (Eds.), *Handbook of Stress and the Brain* (Vol. Part 1, pp. 43-65). Amsterdam: Elsevier.
- Gerra, G., Zaimovic, A., Raggi, M. A., Moi, G., Branchi, B., Moroni, M., et al. (2007). Experimentally induced aggressiveness in heroin-dependent patients treated with buprenorphine: comparison of patients receiving methadone and healthy subjects. *Psychiatry Res*, 149(1-3), 201-213.
- Grawe, K. (2004). *Neuropsychotherapie*. Göttingen: Hogrefe.
- Halasz, J., Liposits, Z., Kruk, M. R. & Haller, J. (2002). Neural background of glucocorticoid dysfunction-induced abnormal aggression in rats: involvement of fear- and stress-related structures. *Eur J Neurosci*, 15(3), 561-569.
- Haller, J., Halasz, J., Mikics, E. & Kruk, M. R. (2004). Chronic glucocorticoid deficiency-induced abnormal aggression, autonomic hypoarousal, and social deficit in rats. *J Neuroendocrinol*, 16(6), 550-557.
- Haller, J., Halasz, J., Mikics, E., Kruk, M. R. & Makara, G. B. (2000). Ultradian corticosterone rhythm and the propensity to behave aggressively in male rats. *J Neuroendocrinol*, 12(10), 937-940.
-

-
- Haller, J. & Kruk, M. R. (2006). Normal and abnormal aggression: human disorders and novel laboratory models. *Neurosci Biobehav Rev*, 30(3), 292-303.
- Haller, J., Mikics, E., Halasz, J. & Toth, M. (2005). Mechanisms differentiating normal from abnormal aggression: glucocorticoids and serotonin. *Eur J Pharmacol*, 526(1-3), 89-100.
- Haller, J., Millar, S., van de Schraaf, J., de Kloet, R. E. & Kruk, M. R. (2000). The active phase-related increase in corticosterone and aggression are linked. *J Neuroendocrinol*, 12(5), 431-436.
- Haller, J., Toth, M. & Halasz, J. (2005). The activation of raphe serotonergic neurons in normal and hypoarousal-driven aggression: a double labeling study in rats. *Behav Brain Res*, 161(1), 88-94.
- Haller, J., van de Schraaf, J. & Kruk, M. R. (2001). Deviant forms of aggression in glucocorticoid hyporeactive rats: a model for 'pathological' aggression? *J Neuroendocrinol*, 13(1), 102-107.
- Heim, C., Ehlert, U. & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology*, 25(1), 1-35.
- Hellhammer, J., Fries, E., Schweisthal, O. W., Schlotz, W., Stone, A. A. & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. *Psychoneuroendocrinology*, 32(1), 80-86.
- Hennig, J., Reuter, M., Netter, P., Burk, C. & Landt, O. (2005). Two types of aggression are differentially related to serotonergic activity and the A779C TPH polymorphism. *Behav Neurosci*, 119(1), 16-25.
- Kim-Cohen, J., Caspi, A., Taylor, A., Williams, B., Newcombe, R., Craig, I. W., et al. (2006). MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis. *Mol Psychiatry*, 11(10), 903-913.

-
- Kirschbaum, C. & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*, *19*(4), 313-333.
- Kolb, B. & Whishaw, I. Q. (1998). Brain plasticity and behavior. *Annu Rev Psychol*, *49*, 43-64.
- Kruk, M. R., Halasz, J., Meelis, W. & Haller, J. (2004). Fast positive feedback between the adrenocortical stress response and a brain mechanism involved in aggressive behavior. *Behav Neurosci*, *118*(5), 1062-1070.
- Kruk, M. R., Westphal, K. G., Van Erp, A. M., van Asperen, J., Cave, B. J., Slater, E., et al. (1998). The hypothalamus: cross-roads of endocrine and behavioural regulation in grooming and aggression. *Neurosci Biobehav Rev*, *23*(2), 163-177.
- Lutzenstroh, B., Birberger, W., Rockbaumer, B. & Thomels, E. (1987). Reactions of the slow wave in the human: a comparison thereof. *Journal of Psychophysiology*, *4*, 349-350.
- Marsman, R., Swinkels, S. H., Rosmalen, J. G., Oldehinkel, A. J., Ormel, J. & Buitelaar, J. K. (2008). HPA-axis activity and externalizing behavior problems in early adolescents from the general population: the role of comorbidity and gender The TRAILS study. *Psychoneuroendocrinology*, *33*(6), 789-798.
- Mason, J. W. (1968). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom Med*, *30*(5), Suppl:576-607.
- McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev*, *87*(3), 873-904.
- Mikics, E., Kruk, M. R. & Haller, J. (2004). Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. *Psychoneuroendocrinology*, *29*(5), 618-635.
- Miller, G. E., Chen, E. & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull*, *133*(1), 25-45.
- Nelson, R. J. & Trainor, B. C. (2007). Neural mechanisms of aggression. *Nat Rev Neurosci*, *8*(7), 536-546.
-

-
- Olvera, R. L. (2002). Intermittent explosive disorder: epidemiology, diagnosis and management. *CNS Drugs*, *16*(8), 517-526.
- Pancheri, P., Picardi, A., Pasquini, M., Gaetano, P. & Biondi, M. (2002). Psychopathological dimensions of depression: a factor study of the 17-item Hamilton depression rating scale in unipolar depressed outpatients. *J Affect Disord*, *68*(1), 41-47.
- Parmigiani, S., Dadomo, H., Bartolomucci, A., Brain, P. F., Carbucicchio, A., Constantino, C., et al. (2009). Personality traits and endocrine response as possible asymmetry factors of agonistic outcome in karate athletes. *Aggress Behav*, *35*(4), 324-333.
- Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., et al. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sci*, *61*(26), 2539-2549.
- Salvador, A., Suay, F., Martinez-Sanchis, S., Simon, V. M. & Brain, P. F. (1999). Correlating testosterone and fighting in male participants in judo contests. *Physiol Behav*, *68*(1-2), 205-209.
- Saplosky, R. M. (2004). *Why zebras don't get ulcers: the acclaimed guide to stress, stress-related diseases, and coping* (3rd ed.). New York: Owl books.
- Sapolsky, R. M., Romero, L. M. & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*, *21*(1), 55-89.
- Steckler, T. (2005). The neuropsychology of stress. In T. Steckler, N. H. Kalin & J. M. H. M. Reul (Eds.), *Handbook of Stress and the Brain* (Vol. Part 1, pp. 25-42). Amsterdam: Elsevier.
- Strüber, D., Lück, M. & Roth, G. (2008). Sex, aggression and impulse control: an integrative account. *Neurocase*, *14*(1), 93-121.
- Taylor, S. P. (1967). Aggressive behavior and physiological arousal as a function of provocation and the tendency to inhibit aggression. *J Pers*, *35*(2), 297-310.
- van de Wiel, N. M., van Goozen, S. H., Matthys, W., Snoek, H. & van Engeland, H. (2004). Cortisol and treatment effect in children with disruptive behavior disorders: a preliminary study. *J Am Acad Child Adolesc Psychiatry*, *43*(8), 1011-1018.
-

-
- van Goozen, S. H., Fairchild, G., Snoek, H. & Harold, G. T. (2007). The evidence for a neurobiological model of childhood antisocial behavior. *Psychol Bull*, 133(1), 149-182.
- van Goozen, S. H., Matthys, W., Cohen-Kettenis, P. T., Buitelaar, J. K. & van Engeland, H. (2000). Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *J Am Acad Child Adolesc Psychiatry*, 39(11), 1438-1445.
- van Honk, J., Tuiten, A. & De Haan, E. (2001). Attentional bias for angry faces: relationships to trait anger and anxiety. *Cognition and Emotion*, 15(3), 279-297.
- Van Praag, H. M. (2001). Anxiety/aggression--driven depression. A paradigm of functionalization and verticalization of psychiatric diagnosis. *Prog Neuropsychopharmacol Biol Psychiatry*, 25(4), 893-924.
- Verona, E., Joiner, T. E., Johnson, F. & Bender, T. W. (2006). Gender specific gene-environment interactions on laboratory-assessed aggression. *Biol Psychol*, 71(1), 33-41.
- Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W. & Wust, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology*, 32(4), 358-366.
- Young, E. A., Abelson, J. & Lightman, S. L. (2004). Cortisol pulsatility and its role in stress regulation and health. *Front Neuroendocrinol*, 25(2), 69-76.

Index of Figures

Figure 0	6
Relationship of the HPA axis and the hypothalamic attack area (HAA) in rodents (Kruk et al., 2004, p. 1067).	
Figure 1	30
Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the provoked group and the non-provoked control group. Values are means \pm SEM.	
Figure 2	31
Correlation of the area under the curve AUC_G of the cortisol awakening response and mean aggressive behavior in the provoked group ($n = 10$). $R^2 = .67$.	
Figure 3	32
Cortisol levels during the experiment in the provoked group and the non-provoked control group. C1, baseline before aggression induction; C2, shortly after aggression induction; C3, about 20 min after aggression induction; C4, at the end of the experiment, about 30 min after aggression induction. Values are original means \pm SEM.	
Figure 4	53
Timeline of the experiment. S = Salivary cortisol sample; P = PANAS state questionnaire.	
Figure 5	56
Mean aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the low and high provocation condition displayed for each gender/treatment condition (high provocation/cortisol/males ($n = 5$); high provocation/placebo/males ($n = 6$); low provocation/cortisol/males ($n = 7$); low provocation/placebo/males ($n = 6$); high provocation/cortisol/females ($n = 6$); high provocation/placebo/females ($n = 5$); low provocation/cortisol/females ($n = 6$); low provocation/placebo/females ($n = 7$)).	
Figure 6	57
Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the low ($n = 26$) and high ($n = 22$) provocation group. Values are means \pm SEM.	

Figure 7	58
Aggressive behavior over the three blocks of the Taylor Aggression Paradigm in the cortisol (n = 24) and the placebo group (n = 24). Values are means ± SEM.	
Figure 8	59
Mean aggressive behavior for males and females in the placebo and cortisol group, respectively (all n = 12). Values are means ± SEM.	
Figure 9	78
Time line for a single trial of the emotional Stroop task.	
Figure 10	83
Mean aggressive behavior of the provoked and the non-provoked group in the three blocks of the Taylor Aggression Paradigm.	
Figure 11	85
Grand average ERP waveforms for the provoked (--) and the non-provoked (-) group averaged over the four facial expressions (happy, neutral, fearful, and angry) and difference maps averaged over the four facial expressions (happy, neutral, fearful, and angry) for the time domains of the P2 (160-200 ms) and P3 (300-400 ms).	
Figure 12	86
Mean P2 and P3 amplitudes (µV) for the provoked and non-provoked participants at Pz electrode site separately for the neutral, happy, angry, and fearful expressions.	

Index of Tables

Table 1	29
Characteristics of the subjects in the provoked group and non-provoked control group (n = 20).	
Table 2	55
Characteristics of the subjects in the four groups: high provocation/cortisol (n = 11), high provocation/placebo (n = 11), low provocation/cortisol (n = 13) and low provocation/placebo (n = 13)	
Table 3	84
Reaction times and bias scores in the emotional Stroop task (means and standard errors).	

Index of Abbreviations

ACC	anterior cingulate cortex
ACTH	adrenocorticotrophic hormone
Ag/AgCl	silver/silver chloride
BPAQ	Buss and Perry Aggression Questionnaire
CRH	corticotrophin-releasing hormone
dB	decibel
EEG	electroencephalography
EOG	electrooculography
ERP	event-related potential
fMRI	functional magnetic resonance imaging
GAM	General Aggression Model
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal
Hz	Hertz
LC-NE	locus coeruleus norepinephrine
LPP	late positive potential
M	mean
mPFC	medial prefrontal cortex
MR	mineralocorticoid receptor
nmol/l	nanomol per liter
OFC	orbitofrontal cortex
P1	first positive component of the event-related potential
P2	second positive component of the event-related potential
P3	third positive component of the event-related potential
PAG	periaqueductal gray
PFC	prefrontal cortex
PSAP	Point Subtraction Aggression Paradigm
SD	standard deviation
SE	standard error
TAP	Taylor Aggression Paradigm
VMPFC	ventromedial prefrontal cortex

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Zudem wurde die Arbeit an keiner anderen Universität zur Erlangung eines akademischen Grades eingereicht.

Trier,

Robina Böhnke

Erklärung zu den Kapiteln II-IV

Bei der in den Kapiteln II und III beschriebenen und in Böhnke et al. 2010a und 2010b veröffentlichten Studien war ich verantwortlich für die Konzeption, Planung, Hypothesenbildung, Datenerhebung, -auswertung, und -interpretation, sowie die schriftliche Ausarbeitung der Manuskripte. Die Koautoren waren mitverantwortlich für die Planung der Experimente und die Datenerhebung (K. Bertsch). Zudem lieferten sie interessante Ideen in der Planungsphase (M. Kruk), sowie Hilfestellung bei der Interpretation (E. Naumann und M. Kruk). S. Richter war an der Datenerhebung beteiligt. Bei der in Kapitel IV beschriebenen und in Bertsch et al. 2009 veröffentlichten Studie war ich mitverantwortlich bei der Planung und Durchführung des Experimentes (dasselbige wie in Kapitel II beschrieben), sowie bei der Ausarbeitung des Manuskriptes unterstützend tätig.

Trier,

Robina Böhnke