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Negative Affect Mediates the Relationship between the Cortisol Awakening Response and Conduct Problems in Boys

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Résumé

Ce mémoire débute avec deux chapitres portant sur les problèmes des conduites et la régulation du stress, notamment sur l'axe hypothalamique-pituitaire-*surrénal* (HPS). Ensuite, la littérature est résumée et nous voyons que les études qui cherchent à établir un lien entre les problèmes des conduites et l'axe HPS ont trouvé des résultats différents et parfois contradictoires. Le chapitre suivant illustre les problèmes méthodologiques qui pourraient expliquer ces résultats différents. Vient ensuite l'étude présentée dans ce mémoire qui cherche à établir un lien entre la réponse cortisolaire à l'éveil (RCE), considérée comme un bon indice du fonctionnent de l'axe HPS, et les problèmes de conduites chez l'enfant. De plus, les émotions négatives ont été associées avec les problèmes des conduites ainsi qu'aux dysfonctions de l'axe HPS, notamment le RCE. L'étude présentée dans ce mémoire cherche aussi à établir si les émotions négatives pourrait être une variable médiatrice dans la relation potentielle entre la RCE et les problèmes des conduites. L'étude révèle que pour les garçons mais pas pour les filles, une RCE réduite est associée avec les émotions négatives, ce qui est successivement associé avec les problèmes des conduites. Le dernier chapitre du mémoire examine les implications théoriques de cette médiatisation et propose également des pistes psychobiologiques pour expliquer les différences sexuelles observées.

Mots clés : l'axe hypothalamique-pituitaire-surrénal, réponse cortisolaire à l'éveil, problèmes des conduites, émotions négatives, différences sexuelles, régulation du stress.

Negative affect mediates the relationship between the cortisol awakening response and conduct problems in boys

Summary

This thesis begins with two chapters which discuss conduct problems and stress regulation, with a focus on the hypothalamic-pituitary-adrenal (HPA) axis. Subsequently, the literature is reviewed and we see that with regards to the relationship between conduct problems and HPA axis activity, the findings are inconsistent. It is possible that methodological considerations underlie the inconsistency found in the literature and the following chapter is concerned with methodology. This is followed by the featured study presented in this thesis that examines the link between the cortisol awakening response (CAR), which is considered a good indicator of HPA axis functioning, and conduct problems in children. Further, negative affect has been linked to both conduct problems and the Cortisol Awakening Response (CAR). Thus it was hypothesized that negative affect acts as a mediator in the cortisol-conduct problems relationship. The featured study found that a reduced CAR was associated with both negative affect and conduct problems, however only in boys and not in girls. Further, the mediation hypothesis was supported in boys. The last chapter in this thesis discusses the implications of this mediation finding for theories of conduct problems as well as proposing some psychobiological mechanisms to explain the sex differences found.

Key words: stress regulation, emotion, hormones and behaviour, sex differences.

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List of Acronyms and Abbreviations

ADHD – Attention-deficit/hyperactivity disorder

AUC – Area under the curve

$CAR_{(i)}$ – Cortisol awakening response (increase)

CD – Conduct disorder

DBD – Disruptive behaviour disorder

DSM-IV-TR – Diagnostic and Statistical Manual of Mental Disorders – text revised

Ed(s). – Editor(s) or edition

GC – Glucocorticoid

HPA – Hypothalamic-pituitary-adrenal

M - Mean

MAOA – Monoamine oxidase A

n – sample size

ODD – Oppositional defiant disorder

PTSD – Post-traumatic stress disorder

Rev. – Revised

SD – Standard deviation

Introduction

The greatest threat to humanity lies in the conduct of men. For millennia, scholars have struggled with the question of what makes people behave badly. Through this research we have come a little closer to answering it. While there is a consensus that the biological mechanisms which regulate stress play an important role in the expression of conduct problems in humans (for reviews see Van Goozen & Fairchild, 2006; Raine, 2002) there is no consensus yet on how this happens. Thus the central research question posed here is *what mechanism accounts for the relationship between stress regulation and conduct problems?*

Hippocrates proposed a description of the anti-social individual around 400 B.C. which after being revived by Pavlov in the 1920s has continued to influence research on this topic through to the present day. One of the characteristics of an anti-social individual was thought to be what we today call neuroticism (see Eysenck, 1964). A neurotic person displays high levels of negative affect.

Negative affect is associated with an important stress regulating mechanism known as the cortisol awakening response (CAR; Polk et al., 2005; Ellenbogen et al., 2006; Steptoe et al., 2007; Stetler, & Miller, 2005) and so the hypothesis tested by this research is that negative affect mediates the relationship between the CAR and conduct problems. This hypothesis was supported and this was the first time that negative affect has been successfully used to explain the relationship between cortisol and conduct problems. This research furthers our understanding of conduct problems by providing a new biological marker for identifying anti-social individuals and by elucidating the mechanism by which the biology is linked to the behaviours.

Overview

This thesis begins with an introductory chapter describing conduct problems, their heterogeneity and similarity, their development and identified risk and protective factors. A prominent theme is the involvement of negative affect as a causal mechanism. In the following introductory chapter the concept of stress and stress regulating mechanisms are discussed. This is followed by a review of the literature concerning the relationship between important stress regulating mechanisms and conduct problems. Subsequent to this, a chapter on methodology describes the issues relating to accurate measurement of those constructs. Then an empirical study is presented which investigates the main research question of this thesis. Finally a concluding chapter summarizes the contributions of this research and discusses its implications for theory and future research.

Chapter 1: An Introduction to Conduct Problems

Overview

This introduction begins by a chapter on conduct problems, discussing why it is important to study them, what they are, theories describing them, their heterogeneity and similarity, their comorbidity with other mental health problems, sex differences in their frequency of expression, how they might develop and be expressed and the relevant risk factors. Potential causal roles for negative affect are discussed where applicable so as to enhance the appreciation of this study's contribution to the understanding of conduct problems. The chapter concludes with a very brief discussion of the role of negative affect. The purpose of this chapter is to provide some background on what is known about conduct problems so that the reader, after reading this and subsequent introductory chapters, will be able to see where the findings of this research fill a gap in our knowledge of the expression of conduct problems.

Why is Studying Conduct Problems Important?

There is a rising incidence of conduct problems which has been well documented in child populations (Sanders, Gooley, & Nicholsen, 2000; Greenberg, Domitrovich, & Bumbarger, 2001). People with conduct problems impose an enormous cost on our society. Even children with sub-clinical levels of conduct problems cost British society more than three times as much as children without conduct problems in terms of public services (Scott, Knaap, Henderson, & Maughan, 2001). Boys with conduct problems are seven times more likely to commit crimes as

adults than boys without conduct problems (Babinsky, Hartsough, & Lambert, 1999). Heckman and Masterov's (2007) economic analysis determined that investing in prevention programs for youth could prove much more cost-effective than investing in policing. Unfortunately, though saving one at-risk youth from a life of crime was estimated to save American society $1.4 million (Cohen, 1998), interventions have had only limited success (Kerns and Prinz, 2002; Greenberg et al.). Hopefully by furthering our understanding of conduct problems we will be able to create better interventions which will allow the potential victims, perpetrators and society as a whole to minimize the enormous costs associated with conduct problems.

This thesis mainly focuses on conduct problems in childhood. Displaying conduct problems is one of the most common reasons why children and adolescents are referred to mental health clinics (Frick, & Silverthorn, 2001) or to residential treatment centers (Lyman, & Campbell, 1996). Focusing on conduct problems in childhood is essential if one hopes to mitigate the costs of these behaviours because by adulthood, these conduct problems may have already seriously affected the mental health of the individual. For instance, children with conduct problems are at risk for developing later depression (among other negative outcomes; Capaldi, 1992; Lahey, Loeber, Burke, Rathouz, & McBurnett, 2002; Patterson, & Stoolmiller, 1991) although the reverse is not true. It is likely that the youth's anti-social behaviour leads to rejection by peers and adults, which in turn fosters depression (Burke, Loeber, Lahey, & Rathouz, 2005). Thus if conduct problems persist into adulthood, serious damage may have already been done.

Why Study Conduct Problems Together Instead of Separately?

Much research on anti-social behaviour in the 20th century has focused on aggression (see Tremblay, 2000) but in the last quarter of that century, researchers often chose to study aggression in conjunction with other anti-social behaviours because their high rates of comorbidity suggested a shared aetiology and developmental course (Coie & Dodge, 1998). Indeed it may be more difficult to understand aggressive behaviours in isolation because they often occur in a context of other problem behaviours such as substance abuse, rebelliousness, delinquency and vandalism (Coie & Dodge). Interestingly, many studies which purported to measure aggression used empirically constructed scales including mainly items that fit better under the rubric of conduct problems (e.g. disobeys teacher, gives dirty looks, makes up stories and lies, does things that bother others, gets in trouble; see Tremblay, 2000) than under the rubric of aggression. Thus even research on aggression is typically actually measuring conduct problems.

While conduct problems cover a broad range of behaviours that range from small offences (lying, violating family curfew, petty theft) to serious offences (assault and sexual assault), the development of juvenile violence begins with conduct problems that are less serious (Lahey & Waldman, 2007). Additionally, all the conduct problems as well as some other mental health problems share certain causal influences (Lahey & Waldman). Thus it is important to consider the full range of conduct problems even when interested in the development of only certain conduct problems such as aggression.

What are Conduct Problems?

Conduct problems can be defined as identifiable behaviours in the individual which fail to conform to societal norms and which encroach upon the rights of others (Frick, 2004; Walker, Kavanagh, Stiller, & Golly, 1998). According to Lahey and Waldman (2007), 'conduct problems' refer to a grouping of anti-social behaviours that includes crimes against persons and property offences (ex: aggression, robbery, vandalism, etc.), status offences (running away from home and truancy), and behaviours that are considered to be symptoms of conduct disorder in the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev. [*DSM–IV–TR*]; American Psychiatric Association, 2000) that typically do not result in arrest (lying, bullying, fighting, violating family curfew, etc.). Certain conduct problems are more prevalent early in life, such as lying and minor aggression (bullying, fighting, and hurting animals) but often decline during school years while other conduct problems including non-aggressive conduct problems (stealing, running away from home, truancy, breaking and entering) and serious forms of aggression (robbery, use of a weapon and forced sex) are more prominent later in life, peaking in adolescence.

Theories of Conduct Problems

Early theories of conduct problems were mainly instinct theories, such as Freud's theory of Thanatos, the death instinct, a suicidal intention that we often direct outwards in the form of aggression (Gillespie, 1971). According to Freud, catharsis, the release of psychic energies, is a way individuals and societies can diminish their drive to aggress. Konrad Lorenz's (1966) theory of aggression was also an instinct theory but, unlike Freud, he saw intra-species aggression as a positive instinct which

promotes strength in a species' gene pool. After observing the behaviour of a variety of species, he concluded that intra-species aggression was essentially a rational behaviour aimed at acquiring or defending resources, such as food, territory and females (without regard to political correctness). This is good because it is in the evolutionary interests of the species that the strong take possession of the resources. Aside from implying that individual differences in the expression of conduct problems are largely genetic and recommending catharsis, which is not effective (Campbell, 1993) as a way to reduce the antisocial passions, instinct theories do not help to explain individual differences in aggression or to formulate interventions. Today, prominent theories that attempt to explain the development and expression of some or all conduct problems include frustration-aggression theory (Berkowitz, 1980), social learning theory (Bandura, 1980) and arousal theories (e.g., sensation-seeking theory; Eysenck, 1964; Zuckerman, 1979; and fearlessness theory; Raine, 1993).

Frustration-Aggression Theory.

According to frustration-aggression theory (Berkowitz, 1980), frustration, which is a perceived threat or hindrance, is the cause of aggression and increasing frustration increases the likelihood of aggression. That elevating levels of frustration increases the likelihood of aggression is something that may seem intuitive to most hockey fans who observe that teams that are losing seem to more often instigate 3rd period fights (Mattesi, 2002). It seems likely that high levels of negative affect would result in higher levels of irritability and frustration, thus increasing the likelihood of aggression and perhaps other conduct problems as well. Inspired by animal studies

which showed that experimentally distressed rats demonstrated aggression (Azrin, Hutchinson, & McLaughlin, 1965; Azrin, Hutchinson, & Hake, 1963), Berkowitz (1993) proposed that pain and distress also trigger aggression. According to Berkowitz's model (1993), high levels of pain and distress trigger the fight or flight system and the more intense the animal's resulting negative affect is, the more likely aggression becomes.

Social Learning Theory.

Noticing the large differences in levels of conduct problems across cultures, Bandura (1980) decided that they are primarily a learned response. Social learning theory states that behaviour is learned by modeling others (Bandura, 1977). In a famous experiment, when children were shown a confederate playing aggressively with a blow up doll named Bobo, they were afterwards more likely to exhibit aggressive behaviours towards Bobo, especially if they had seen the aggressive confederate being rewarded (Bandura, 1980). Returning to the hockey example, after extensive research, Smith (1988) concluded that junior hockey players modeled the aggressive acts of professional players, believing, often rightly so, that it will get them recognition, respect and other rewards. However, modeling theories are limited in that they do not account for the fact that, despite an increase in exposure to violence in the media, developmental studies have shown that physical aggression declines over time (Nagin & Tremblay, 1999).

Arousal Theories.

Low levels of physiological arousal are the most consistent biological correlate of conduct problems (Raine, 1993). Fearlessness theory (Raine) postulates that low levels of arousal are characteristic of fearless individuals who remain calm during stressful situations. These individuals do not fear the consequences of their actions and thus feel free to engage in antisocial behaviour that would be too frightening for normal people. Thus fear protects individuals from expressing antisocial behaviour by inhibiting their behaviour in circumstances where such behaviour could lead to negative consequences (e.g., social disapproval, punishment, jail, risk of injury). Sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979) postulates that low levels of arousal are perceived as an aversive state. That is, under-aroused individuals are bored and unhappy. This leads them to engage in risky, often antisocial behaviour in order to raise their levels of arousal to a more optimal level. Thus in this theory negative affect is a causal factor which motivates individuals to seek stimulation to reduce such unpleasant emotions. Of these four prominent theories, two (frustration-aggression theory and sensation-seeking theory) include causal roles for negative affect. Interestingly, by combining elements of sensation-seeking theory with frustration aggression theory, a new theory is possible whereby low levels of arousal produce negative affect, which, in turn, increases frustration, thereby leading to anti-social behaviour.

Heterogeneity and Similarity of Conduct Problems

Oppositional Defiant Disorder and Conduct Disorder.

There is considerable heterogeneity as well as similarity among conduct problems. For instance, a distinction between different types of conduct problems is made in the *DSM–IV-TR's* diagnostic categories of oppositional defiant disorder (ODD) and conduct disorder (CD). CD is considered more serious and consists of aggressive (e.g., fighting, bullying) and destructive (e.g., setting fires, vandalism) behaviours as well as thieving (e.g., breaking and entering) and major disobedience (e.g., running away from home). In contrast ODD is considered rather benign by some (Loeber, Stouthamer-Loeber, VanKammen, & Farrington, 1991) and consists of minor disobedience (e.g., not complying with rules and requests), hostile (e.g., losing temper) and other negative behaviours (e.g., deliberately doing things that annoy other people, blaming others for own mistakes). Most youth with CD display ODD behaviours as well (Lahey & Loeber, 1994). In contrast, most youth with ODD do not show the more serious conduct problems associated with CD (Lahey & Loeber). However they have an approximately four-fold greater risk of developing CD than non-ODD youth (Cohen, & Flory, 1998). Over the course of a three year longitudinal study of clinic-referred boys, 82% of the new cases of CD had a diagnosis of ODD the previous year (Lahey & Loeber). Thus the distinction between ODD and CD may be more of difference of degree and age rather than one of kind. CD behaviours are typically observed at a later age than ODD behaviours (Kelley, Loeber, Keenan, & DeLamatre, 1997; Loeber, Keenan, & Zhang, 1997; Loeber, Farrington, Stouthamer-Loeber, & Van Kammen, 1998). It is likely that children with the most severe conduct problems at one age preserve their relative rank despite their absolute levels

of conduct problems varying over time (see Farrington, 1997). However, it is unclear to what extent this continuity is typical of girls where late onset CD is more common (Silverthorn, & Frick, 1999) leading some (Burke, Loeber, & Birmaher, 2002) to speculate that ODD may not precede CD as often in girls who may have an alternate pathway to CD.

Overt and Covert Conduct Problems.

In addition to the separation of ODD from CD, other distinctions have been made among conduct problems. A meta-analysis on more than 60 published factor analyses including more than 28,000 children and adolescents found that conduct problems could be described by two bipolar dimensions (Frick et al., 1993): an overt-covert dimension and a destructive-nondestructive dimension. Examples of related behaviours include aggression for the overt-destructive category, property offences such as vandalism for the covert-destructive category, opposition for the overt-nondestructive category and status offences such as under-age drinking for the covert-nondestructive category.

There may be important differences between the aetiologies of conduct problems in the different categories with a twin study showing higher heritability of destructive than non-destructive conduct problems (Simonoff, Pickles, Meyer, Silberg, & Maes, 1998). On the other hand, non-destructive and destructive behaviours may share a similar aetiology, with more destructive behaviours evidenced in more serious cases. This can just as easily account for the heritability findings since in a more serious case one would expect to find more genetic risk factors than in a less serious one. As with the ODD-CD distinction, a developmental

progression between categories seems to occur here as well. Behaviours from the overt-nondestructive category, such as non-compliance, appear early and persist in individuals with conduct problems (see Chamberlain & Patterson, 1995; Loeber et al., 1993; McMahon & Forehand, 2003). It seems likely that covert behaviours would tend to appear later as anti-social children become more sophisticated and serious destructive behaviours would tend to appear later as they gain power.

Physical and non-Physical Aggression.

There are some important distinctions among the aggressive behaviours. For instance, there are both physical (e.g., hitting) and non-physical (e.g., insulting and e.g., gossiping) aggressive behaviours (Infante, & Wigley, 1986; Lagerspetz, Bjorkqvist, & Peltonen, 1988; Vaillancourt, 2005). Physical aggression tends to decrease in frequency during childhood and adolescence (Nagin, & Tremblay, 1999) and is more common in boys (Broidy et al., 2003). Non-physical aggression tends to increase in frequency during childhood and adolescence and is more common in girls, especially the social type (Underwood, 2003). Both the age and sex differences in aggression subtype preference may be partially explained by age and sex differences in 1) verbal and social skills which are essential for effective verbal and social aggression and 2) socialization mechanisms by which girls and older children learn that physical aggression is ineffective and costly compared to social aggression (Côté, 2007).

Proactive and Reactive Aggression.

Another important distinction among aggressive behaviours is that some are reactive in nature whereas others are proactive in nature (see Dodge & Coie, 1987). Proactive aggression is controlled and associated with low arousal (van Bokhoven et al., 2005). It is the type of aggression that characterizes the school-yard bully (Camodeca, & Goossens, 2005) and the 'cold-blooded' psychopath (Blair et al., 2004). It is also described as instrumental aggression because proactive aggressive behaviour usually serves to dominate or exploit others with the aim of procuring valuable resources (Dodge & Coie). Reactive aggression, in contrast, is associated with disinhibition, affective instability and high levels of arousal (van Bokhoven et al., 2005) and is a response to actual or perceived provocation or threats (Brengden, Vitaro, Tremblay & Lavoie, 2000). It is characteristic of the retaliatory aggression of someone who feels victimized or insulted.

The distinction between proactive and reactive aggression has been empirically validated (Dodge & Coie, 1987; Poulin & Boivin, 2000; Vitaro, Brendgen, & Tremblay, 2002), however, these two subtypes of aggression often co-occur with correlations ranging from .30 to .70 in school age samples (Poulin & Boivin). Interestingly, very few purely proactively aggressive children are found (Barker, Tremblay, Nagin, Vitaro, & Lacourse, 2006) with nearly all highly proactively aggressive children displaying at least moderate amounts of reactive aggression. Yet many purely reactively aggressive children are found. These two types of aggression are hypothesized to be associated with distinct brain areas and systems (Peterson & Shayne, 2005). While aggression in general is associated with a lack of self control (as are other conduct problems; Caspi et al., 1995; Shoal et al.,

2003), proactive aggression is especially associated with a need to control others (Winstok, 2009). Thus, while the aetiology of proactive aggression shares elements with reactive aggression and other conduct problems, it also has exclusive predisposing factors, leading Peterson & Shayne to describe it as 'a different kind of animal altogether'.

Comorbidity

Youth who display high levels of conduct problems are also likely to display comorbid mental health problems such as attention-deficit/hyperactivity disorder (ADHD) anxiety disorders and depression (Angold, Costello, & Erkanli, 1999; Lahey, Miller, Gordon, & Riley, 1999). ADHD is the condition most commonly co-occuring with conduct problems. Waschbusch's (2002) meta-analysis found that 36% of boys and 57% of girls with conduct problems had comorbid ADHD. Interestingly, comorbid ADHD usually signals a more severe and chronic form of conduct problems (see Waschbusch, 2002).

The high rate of co-occurrence among conduct problems and other mental health problems is likely due to shared dispositional and cognitive profiles such as high negative affect (Sanson, & Prior, 1999) and low intelligence (see Hinshaw, 1992; Hogan, 1999; Waschbusch, 2002). Indeed, Lahey and Waldman (2007) posit that negative emotionality acts as a non-specific dispositional risk factor which fosters the development of many types of problems which often co-occur. If true, this might explain why having comorbid ADHD is associated with a more virulent form of conduct problems in that having a more affectively negative disposition could both increase the number of mental health problems present and their severity; so having

more problems would also predict having more severe problems because of the common non-specific dispositional cause.

Sex Differences

It is commonly observed that males exhibit more conduct problems than females. By school entry, boys display significantly more conduct problems than girls (Keenan & Shaw, 1997; Moffitt, Caspi, Rutter, & Silva, 2001). In a longitudinal study of Columbia county third graders of 1960 it was found that by age 30, 20% of males had criminal records compared to only 5.8% of females (Huesmann, Dubow, & Boxer, 2009). Girls may be more likely to have dispositions and intellectual capacities amenable to proper socialization than boys. For instance, because girls tend to have better language skills during the crucial toddler years (Sanson, Smart, Prior, & Oberklaid, 1993), they may be easier to socialize (Keenan, & Shaw, 1997). Also, girls tend to show higher levels of empathy and guilt during toddler-hood through adolescence (Keenan, Loeber, & Green, 1999; Keenan, & Shaw; Zahn-Waxler, Robinson, & Emde, 1992) which ought to inhibit their antisocial behaviour (Kochanska, Barry, Jimenez, Hollatz, & Woodard, 2009).

Development

Conduct problems begin about as soon as children develop the capacity to display them. Nearly half of all toddlers hit, kick, intentionally break things, take others toys, lie and resist the authority of adults from the time they can walk and talk (Tremblay et al., 1999). Most children learn to inhibit these behaviours over the course of their development. These findings contradict the still highly influential

'noble savage' hypothesis (Rousseau, 1762/1979) which proposes that socialization corrupts the pure individual, born as a blank slate and learning to sin. Instead the facts tend to support the rival hypothesis in this age-old debate - that children are born unruly and some are successfully socialized into civilized adults (Hobbes, 1651/1958); so rather than looking at the development of conduct problems through a social-learning perspective (Bandura, 1977) whereby children learn to imitate the anti-social behaviour of others, it may be more accurate to think of children failing to 'unlearn' developmentally early behaviours, such as fighting, at an appropriate age (Tremblay, 2000). Likely, however, both the learning of new anti-social behaviours and the failure to unlearn old ones contribute to conduct problems later in life.

Developmental Trajectories.

In order to understand the development of conduct problems, Nagin and Tremblay (1999) applied the concept of developmental taxonomy (Moffit, 1993) to a longitudinal study of children's conduct problems in order to group individuals into discrete developmental courses. Although many studies have focused on developmental trajectories of aggression and other conduct problems (Nagin & Tremblay; Maughan, Pickles, Rowe, Costello, & Angold, 2001; see also Broidy et al., 2003 and Moffit, 2007) the Dunedin Study has measured conduct problems in males over a comparatively long time: from age 7 to age 26. A full description of the Dunedin Study, which provides the trajectory membership statistics for the following section, can be found in Moffitt et al. (2001).

Five distinct trajectories have been identified that describe the development and persistence of conduct problems (Moffit, 2007). First, a small group (11% of the

total sample) has been identified that consists of abstainers from antisocial behavior. These individuals tend to be extremely overcontrolled, fearful, interpersonally timid, socially inept and socially excluded although they are often unusually good students (Moffit, Caspi, Dickson, Silva, & Stanton, 1996). Second there are those (41% of the total sample) who consistently display very low levels of conduct problems. These individuals constitute the 'mainstream' and this group is not heavily studied. Third, there are individuals (14% of the total sample) who only display conduct problems during adolescence. This is thought to be caused more by social and cultural factors than by individual or family risk factors (see below; Moffitt, 1993). Indeed, research shows that during adolescence, unlike during other life periods, anti-social behaviour is socially and culturally endorsed while being a good student is lowered in status (Bukowski, Sippola, & Newcomb, 2000; Luthar, & McMahon, 1996; Rodkin, Farmer, Pearl, & Van Acker, 2000). The fourth and smallest group is the life-course persistent trajectory, whose members (7% of the total sample) display high levels of conduct problems from childhood to adulthood. These individuals are typically characterized by more individual and family risk factors (see these sections of the text below) than previously mentioned groups (Moffitt, 2007). Finally, there is a group (21% of the total sample) with early onset conduct problems who are similar to the life-course persistent group but appear to ameliorate during adolescence. This group has been called "childhood-limited", "recoveries" and "low level chronic offenders" depending on how long the cohort is followed. Typically, studies that followed these children into adulthood found that they continued to display problem behaviour as adults, however in a more intermittent fashion than the life-course persistent group (Moffitt, 2007). It is possible that these individuals desist from anti-social behaviour

to some extent during adolescence because they have off-putting personal characteristics that exclude them from association with delinquent peer groups (Moffitt, 2007). However, other protective and risk factors may also play a role.

Family Risk Factors

Family factors receive a lot of attention when it comes to the development of conduct problems (see Farrington, 2007). Children with antisocial parents tend to be antisocial themselves (Johnson, Smailes, Cohen, Kasen, & Brook, 2004). Intergenerational transmission of conduct problems may be mediated by a variety of factors, including shared community risk factors, shared genetic risk factors and poor parenting (see Farrington, Jolliffe, Loeber, Stouthamer-Loeber, & Kalb, 2001). While the genetic contribution to conduct problems is likely complex, a single gene, monoamine oxidase A (MAOA), which metabolizes the neurotransmitter serotonin, has received much attention, being related to both aggressiveness in mice (Shih et al., 1999) and humans (Brunner, Nelen, Breakefield, & Ropers, 1993) although the relationship is still controversial in the latter case (Parsian, & Cloninger, 2001). A meta-analysis by Rhee and Waldman (2002) involving 42 independent twin samples and 10 adoption samples estimated the heritability of conduct problems to be 0.47; thus, while genes are important, other factors clearly also play a role. Harsh parental punishment, large family size, poor parental supervision, and other family risk factors have been identified (Farrington, 2007). Parental conflict predicts children's later conduct problems (Buehler et al., 1997) as does parental abuse and neglect (Widom, 1989; Lang, Klinteberg, & Alm, 2002). Interestingly, in their meta-analysis, Kim-Cohen et al. (2006) found that child maltreatment only predicted later conduct

problems for males with low MAOA activity but not for males with high MAOA activity.

Individual Risk Factors

Dispositional Risk Factors.

Dispositional risk factors reflect individual differences in predisposition towards developing conduct problems and are assumed to be biologically influenced early in life, such as through genes and the pre-natal environment. A difficult temperament (Maziade et al., 1985), impulsivity (Lengua, Wolchik, Sandler, & West, 2000), negative affect (Belsky, 2004; Belsky, Hsieh, & Crnic, 1998) and lack of positive affect (Lengua et al.) have all been associated with conduct problems. Supporting fearlessness theory (Raine, 1993), Shaw, Gilliom, Ingoldsby and Nagin (2003) found that among Americans, observed fearlessness at age 2 distinguished members of a developmental trajectory with chronically high levels of conduct problems from all other trajectories. In support both fearlessness and sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979), Raine, Reynolds, Venables, Mednick and Farrington (1998) found that stimulation-seeking as well as fearlessness at age 3 distinguished aggressive from non-aggressive Mauritians at age 11. Additionally, impulsivity measured during kindergarten predicted self-reported delinquency at age 13 among Canadian boys (Tremblay, Pihl, Vitaro, & Dobkin, 1994). Finally, Caspi et al. (1995) studied New Zealanders and found that those who demonstrated a ‘lack-of-control temperament’ at age 3 displayed more conduct problems between ages 9 and 11, more conduct disorder between ages 13 and 15 and more violent convictions at age 18 (Henry, Caspi, Moffitt, & Silva, 1996).

Personality Risk Factors.

Important personality factors may contribute to the expression of conduct problems. Hyperactivity, impulsiveness, poor behavioural control, risk-taking and low intelligence have been identified as risk factors and may be caused by deficits in executive functioning which is controlled by the frontal lobes of the brain (Farrington, 2007). A meta-analysis by Morgan and Lilienfeld (2000) of thirty-nine studies, with a total of 4,589 participants, found that participants who displayed antisocial behaviours performed significantly worse than comparison groups in tests measuring executive functioning (combined and weighted Cohen's d = 0.062) (Cohen, 1992). Different types of anti-social behaviour yielded somewhat different effect sizes; however these studies used different comparison groups rendering interpretation difficult (Morgan & Lilienfeld). Unfortunately, the authors were not able to examine the effects of ADHD, which is often found to co-occur with conduct problems (Barkley, Guevremont, Anastopoulos, Dupaul, & Shelton, 1993) and is also associated with neuropsychological deficits (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). However, studies in children demonstrate an association between physical aggression and executive functioning that remains after controlling for hyperactivity (Séguin, Nagin, Assaad, & Tremblay, 2004) and ADHD (Giancola, Mezzich, & Tarter, 1998; Séguin, Boulerice, Harden, & Tremblay, 1999). Similar results have also been found in studies of bullying (Coolidge, DenBoer, & Segal, 2004).

While some have suggested that neuropsychological impairments may be more present among individuals characterized by the early onset/persistent trajectory

of conduct problems, Raine et al. (2005) found that the childhood limited group was just as impaired as the persistent group. This means that other factors besides neuropsychological impairment likely explain the persistence of conduct problems beyond childhood.

Biological risk factors.

Several biological risk factors for conduct problems have been identified (Raine, 2002). Raine (1993) observed that one of the most replicable findings in the literature is that people with conduct problems tend to have low resting heart rates (see also Lorber, 2004). Theoretically, low heart rate indicates low autonomic arousal and/or boredom. Sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979) postulates that low autonomic arousal is perceived as negative affect (boredom and unhappiness) and can lead individuals to seek sensation and take risks, often antisocial ones, in order to increase their arousal. Additionally, high heart rate is associated with anxiety, behavioural inhibition and fearfulness (Kagan, 1989) which tend to inhibit violence (Farrington, 2007) and may thus be a protective factor in regards to the development of conduct problems (Raine, 2002). Low heart rate measured at age 11 predicted convictions for violence and sexual offences up to age 21 (Wadsworth, 1976). Interestingly, while boys who had experienced living in a broken home before age 5 tended to display low heart rates, low heart rate was only a predictor of violent or sexual offenses among boys who came from intact homes (Wadsworth). This is concordant with the social-push hypothesis (Raine, 2002; Raine, & Venables, 1981) which predicts that biological risk factors for anti-social behaviour are easier to detect when psychosocial risk factors are absent. Birth

delivery complications may also play a role in the development of conduct problems (Kandel, & Mednick, 1991), especially when a parent had a history of psychiatric illness (Brennan, Mednick, & Mednick, 1993). Indeed, interactions between biological and psychosocial factors are quite common (Raine, 2002; Raine, Brennan, & Farrington, 1997).

Interactions between risk factors

Dodge and Sherrill (2007) conducted a meta-analysis on studies measuring both individual and environmental risk factors for anti-social behaviour. They identified two true interaction effects that might characterize the relationship: 1) that high levels of either individual or environmental risk factors are sufficient to lead to anti-social outcomes and that having high levels of both would not make much additional difference (Mednick, & Christiansen, 1977) and 2) that children with the highest individual risk for anti-social outcomes are the most susceptible to environmental risk factors (see the organismic-specificity hypothesis, Wachs, & Gandour, 1983; also see the biological-sensitivity to context hypothesis, Belsky, 2004), have the most variable life-course outcomes and thus would most benefit from targeted interventions.

Studies that have examined biological risk factors for conduct problems such as birth complications (Raine, 2002) have found that an adverse environment increases risk the of developing conduct problems much more among children with serious birth complications than among those with easy births (Arsenault, Tremblay, Boulerice, & Saucier, 2002; Hodgins, Kratzera and McNeil, 2001). In a study of three-year-old boys, Belsky et al. (1998) found that hostile parenting was more

strongly related to conduct problems among boys high in negative affect, an individual risk factor, than among boys low in negative affect and Belsky (2004) reported that low maternal sensitivity was more strongly related to child disruptive behaviour problems in children with high levels of negative affect. Similarly, Lengua et al. (2000) found that maternal rejection was significantly related to conduct problems in 9 to 12-year-olds but only for the children who were low in positive affect. These studies support the hypothesis that environmental influences are most important for children who already possess biological and dispositional risk factors. Unfortunately, environmental and dispositional risk factors are often positively correlated (Dodge & Sherrill, 2007) meaning that the children most in need of a supportive environment are the least likely to be in one.

Negative Affect

As mentioned above, negative affect has been associated with conduct problems in numerous studies (Belsky, 2004; Belsky, Hsieh and Crnic, 1998; Loney et al., 2006; Séguin, Arseneault, Boulerice, Harden, & Tremblay, 2002; Schmitz et al., 1999; Gjone, & Stevenson, 1997). Based on studies of similar traits, it is likely that negative affect is influenced moderately by both genes and environment (Lahey & Waldman, 2007). Aversive states in general are thought to lead to aggressive behaviour (Anderson, & Bushman, 2002) and experiencing negative affect in particular is also likely to lead to irritability, talking back and rebelliousness (Susman et al., 1987). Negative affect plays a role in both the frustration-aggression theory (Berkowitz, 1980; also see Berkowitz, 1993) of aggression and the sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979) of conduct problems. Additionally,

children high in negative affect are also likely to be low in self-control (a predictor of conduct problems) because powerful emotions can overwhelm even the most skilful self-regulators (Gross, & Muñoz, 1995). Thus negative affect seems to play an important causal role in the expression of conduct problems.

Summary

Conduct problems refer to a grouping of anti-social behaviours that includes crimes against persons and property offences (ex: aggression, robbery, vandalism, etc.), status offences (running away from home and truancy), and behaviours that are considered to be symptoms of conduct disorder in the DSM-IV that typically do not result in arrest (lying, bullying, fighting, violating family curfew, etc.). Prominent theories that attempt to explain the development and expression of some or all conduct problems include frustration-aggression theory, social learning theory and arousal theories (e.g., sensation-seeking theory and fearlessness theory). Of these four theories, two (frustration-aggression theory and sensation-seeking theory) include causal roles for negative affect.

There is considerable heterogeneity as well as similarity among conduct problems. Distinctions among conduct problems include oppositional defiant and conduct disorder symptoms, destructive, non-destructive, overt and covert conduct problems, and aggression subtypes (physical, verbal and social; proactive and reactive). Many of these behaviours are found together and likely share similar aetiologies and predisposing factors, with the possible exception of proactive aggression which may be somewhat distinct. In general, much of the heterogeneity seems explainable by the fact that different conduct problems appear in different

frequencies at different ages and may reflect different degrees of severity of conduct problems as well as gender differences in pre-disposing factors. Co-occurence of conduct problems as well as high rates of comorbidity with other mental health problems likely reflect common predisposing factors, such a as tendency to experience negative affect.

Conduct problems begin about as soon as children develop the capacity to display them. Nearly half of all toddlers hit, kick, intentionally break things, take others toys, lie and resist the authority of adults from the time they can walk and talk. Most children learn or are socialized to inhibit these behaviours over the course of their development. However, while desisting from conduct problems is common, in some individuals high levels of conduct problems persist past childhood and adolescence. When the social environment is relatively benign, this may be explained by chronically antisocial individuals having more biological risk factors and less protective ones than desisting individuals, as would be predicted by the social push hypothesis. Alternatively, individuals in both trajectories may have high levels of biological risk factors and the different paths they take may be due to the different social environments they are exposed to.

Multiple risk factors for conduct problems have been identified, including family risk factors such as genetic risk, poor parenting and bad neighbourhoods. In addition, individual factors such as poor executive functioning, sensation-seeking, risk-taking, low empathy and negative affect play a role. Interactions between biological and other risk factors are common; for example, high biological risk typically increases sensitivity to other risk factors. Thus, children with a tendency to display negative affect likely have a greater risk of developing conduct problems than

less affectively negative children when they are also exposed to poor parenting, bad neighbourhoods and other risk factors.

Chapter 2: Stress Regulating Mechanisms, the HPA Axis and Cortisol

Overview

This introduction concludes with a chapter on stress regulating mechanisms, the HPA axis and cortisol. Topics covered include early notions of stress, theories of stress, the stress response, homeostasis and allostasis, stress hormones, why they are released and how they are regulated. Different components of HPA axis activity are reviewed with a focus on cortisol secretion. Finally, the effects of these hormones are discussed with emphasis placed on cortisol's regulation of physiological and psychological processes. The purpose of this chapter is to give the reader background information on psychoneuroendocrine mechanisms that regulate stress, especially the hormone cortisol, which is the main product of the HPA axis in humans. This is done so that the reader can appreciate the significance and theoretical implications of this study's finding that negative affect mediates the relationship between conduct problems and a distinct component of HPA axis activity.

Early Notions of Stress

Although perhaps not as popular a term as it is today, the concept of psychological stress existed in Western culture prior to the 20th century. Lazarus (1993) reports that usage of the term 'stress' can be found as early as the fourteenth century (Lumsden, 1981, cited in Lazarus) when it was used to refer to hardship and adversity. Early use of a broader conception of stress that includes such stresses as the mental 'stress of learning' is evident in traditional Lao culture. However, in Lao Buddhism, which has been a major influence in highly religious Laos for hundreds of

years, stress is considered the antithesis of good karma and to be avoided at all costs, with even education seen as unhealthily stressful (McClements, 2000). Perhaps unsurprisingly, Laos is one of the world's least developed countries (Ministry of Foreign Affairs of Japan, 2004). However, perhaps surprisingly, considering the typically high rate of violent crime in developing countries, Laos has less crime than even the U.S., especially less violent crime, despite a generally ineffective and un-reactive police force (Overseas Security and Advisory Council, 2008). This suggests that stress may play a role in the expression of conduct problems.

The Stress Response

Hans Selye, who is credited with popularizing the concept, defined stress as a "*nonspecific response of the body to a demand*," (Selye, 1946). This response, which is made up of interrelated adaptive reactions, is termed the 'General Adaptation Syndrome' and is divided into three phases: the alarm reaction, the stage of resistance, and finally the stage of exhaustion (Selye, 1950). In the alarm stage, the organism rallies its resources to meet the demand. However, if the cause of stress is not removed, the organism then passes into the stage of resistance, in which more resources are rallied to the cause of resisting a long-lasting stress. Finally, once the organism's coping resources have been expended, it enters the stage of exhaustion. The purpose of this whole process is to allow the organism to cope with an external demand and then to restore homeostasis (Selye, 1950).

Homeostasis and Allostasis

The term homeostasis, which is Greek for 'to stand equally', was coined by Walter Bradford Cannon (Cannon, 1935) and refers to the process of keeping a system's internal environmental parameters constant. In biology, the system is a living organism and internal environmental parameters can be such things as body temperature or blood sugar level. Homeostasis can be based on negative feedback, a process in which a change in an internal environmental parameter, such as raised blood sugar, sends a feedback signal that inhibits further change in that direction, for instance, by inhibiting further production of glucagon, a hormone which raises blood sugar levels. A homeostatic response may also involve releasing insulin to further lower blood sugar and return it to its original level. Note that the homeostatic response attempts to counter change whether that change results from an external source, such as consuming a sugary drink, or an internal source, such as catecholamine-mediated blood sugar increase.

In contrast to homeostasis, which attempts to counter change, allostasis, which translates from Greek as 'remaining stable by being variable', is about adaptation. In *Beyond the Stress Concept: Allostatic Load* (Lupien et al., 2006), the authors argue that the same processes, which attempt to cope with stressors and restore homeostasis during stress, lead to a build-up of allostatic agents such as hormones, neurotransmitters and oxidants. The degree of this build up of chemicals is known as the allostatic load. During and after stress, if the organism cannot effectively cope with stressors and restore homeostasis, the allostatic load will increase, resulting in 'wear and tear'. The allostatic process is thought to underlie all stress-related long-term change.

Theories of Stress

While Selye's (1946) theory of stress defines it as a *reaction* to demands placed on the organism, it has also been described as a *threat* (Spielberger & Reheiser, 1994) and as a *process* (Lazarus, 1991). Spielberger and Reheiser (1994) describe stress as the object or condition perceived as threatening; in other words, the stressor, of which they describe many different types. Lazarus' (1991) transactional process model emphasized the cognitive evaluation of the situation which both influences and is influenced by the organism's reaction.

Good Stress and Bad Stress.

According to Selye (1974), stress is divided into eustress, which is Latin for good stress, and distress, which is Latin for bad stress. While eustress is adaptive, for example: the stress of having to meet a deadline causing you to work harder and meet that deadline, distress is maladaptive, for example: the stress of having to meet an impossible deadline causing you to have a nervous breakdown.Whether a stress is good or bad depends on how you perceive the situation, demonstrating the importance of Lazarus' (1991) cognitive appraisal. The determining appraisal is how you evaluate the discrepancy between the demands of a certain situation and the resources you have, be they physical, material, psychological, spiritual or social, to deal with those demands. For instance, if you perceive your resources as equal to or greater than the demands of a situation, you will experience eustress; however, if you believe your resources to be inadequate, you will experience distress.

The Stress-Performance Curve.

The stress-performance curve is based on an inverted U-shaped stress-performance hypothesis (see Henry, 1993) with stress on the X-axis and performance on the Y-axis. Optimal performance in a given physical or mental task is achieved with maximal eustress and minimal distress, which is represented by an intermediate level of overall stress. Reducing eustress, by reducing overall stress to below the optimal level, will lead to reduced performance as will increasing overall stress above the optimal level, which results in increasing levels of distress.

Consequences of Stress

Stress is thought to be a cause of premature ageing and age related diseases and this is supported by recent evidence (Liu & Mori, 1999). People have long suspected that exposure to stress has many negative biological consequences, including hair loss (Arck et al. 2001). Many such suspicions have been supported; however the hair loss hypothesis has not received support (Arck et al., 2001). Recently, we are also becoming aware of the role stress plays in the aetiology of psychological ailments. For instance, exposure to acute stress can lead to post traumatic stress disorder **(**Harvey, & Bryant, 1998**)** and exposure to chronic stress can lead to burn-out (Cordes & Dougherty, 1993).

Stress Hormones

The stress response makes use of stress hormones to effectuate physiological and psychological changes that allow the organism to cope with stressors. Hormones are chemical messengers that carry information through the blood to a variety of

target receptors throughout the body and brain and are thus effective for producing widespread and varied changes in an organism. Important stress hormones include the hormone adrenaline, as well as noradrenaline and the glucocorticoids (GCs). Noradrenaline and adrenaline which are categorized as catecholamines, are produced in the adrenal medulla which is triggered by sympathetic nervous system activation (Haller, Makara, & Kruk, 1998) and GCs are produced in the adrenal cortex and are the end product of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Both of these systems and their hormones have been so effective in keeping organisms alive that they have been preserved through hundreds of millions of years of evolution, originating before life moved out of the oceans **(**Bonga, 1997**).** Although adrenaline, noradrenaline and the GCs are all considered 'stress hormones', many important differences exist. For instance, noradrenaline and adrenaline are released quickly when stressful situations call for a specific energetic response such as fighting or fleeing (Haller et al.) whereas the HPA axis takes a bit more time, is known to respond to a variety of physical, emotional and social stressors and is thought to be most responsive when the stimulus is novel, unpredictable and uncontrollable (Dickerson & Kemeny, 2004).

Short-term physiological effects of the stress induced catecholamines are aimed at preparing the organism for quick action and include increased heart rate, blood pressure and blood sugar as well as decreased blood flow to the gastrointestinal tract, which is not relevant to fight or flight demands (Haller et al., 1998). Short-term physiological effects of GCs serve to increase the energy available to the organism while it deals with the stressor and include glucogenesis, lipolysis, proteolysis, insulin resistance, inflammation reduction and immune system inhibition (Henry, 1993).

Essentially, the release of stress hormones prioritizes the short-term, action-oriented needs of the organism by expending energy reserves and diverting resources away from systems which support medium and long term needs such as the digestive and immune systems.

Stressor-Specific Hormone Release

Although Selye (1946) originally thought of stress as a general response to situational demands, which presupposes a uniform response to a variety of stimuli, the biological as well as the behavioural response clearly varies depending on the organism's appraisal of the situation (Henry, 1993). A situation perceived as within one's capacity to handle will elicit an active coping response, coinciding with the release of noradrenaline (Henry). If the organism does not perceive the situation to be within its control, it switches to a passive coping response and the noradrenaline/adrenaline ratio decreases as adrenaline levels rise (Henry). At higher levels of situational uncertainty and distress, the HPA axis reacts and adrenocorticotropic hormone and GC levels rise (Dickerson & Kemeny, 2004; Henry). Thus, differently perceived stressors will result in different specific stress responses, which are differentially associated with the principal stress hormones and their related coping styles.

The HPA Axis

When a stressor is encountered, the brain's hypothalamus releases corticotrophin-releasing hormone, which travels through the blood vessels of the hypothalamic-pituitary portal system, which is inside a tiny structure called the

infundibulum, latin for funnel, connecting the hypothalamus to the pituitary gland. Corticotropin-releasing hormone in the pituitary gland triggers the release of adrenocorticotropic hormone, which then travels through the blood to the adrenal cortex where it in turn triggers the release of GCs, cortisol being the primary GC in humans. The HPA axis has a negative-feedback system in which the presence of each hormone inhibits the upstream (prior in the sequence) release of hormones. This is a homeostatic system which is supposed to help stabilize and reduce levels of stress hormones after the stressor has been removed. HPA axis activity can also be up or down-regulated in a variety of ways, for example, exposure to stress could cause an increase in the number of receptors which mediate the above-mentioned negative feedback thus reducing the magnitude of hormone release during the next stress response (a type of physiological habituation to stress).

Components of HPA Axis Functioning

Reactive and Baseline Cortisol.

When cortisol is secreted in reaction to a stressor, as described above, the ensuing change in cortisol levels, which takes about 17-32 minutes to detect in saliva (see Stansbury & Gunnar, 1994 and Gunnar & White, 2001), is known as cortisol reactivity. This is similar to the concept of heart-rate reactivity and heart rate will also change in response to a stressor. However, just like the heart, which has a baseline resting heart rate that can be observed in the absence of stressors, the HPA axis also has a baseline level of cortisol secretion. Baseline cortisol secretion follows a diurnal rhythm with a typically 10-fold variation in concentration from 8 A.M. to 8 P.M. in the saliva of 12- to 18-year-olds (Goodyer, Park, Netherton, & Herbert, 2001). This

daily rhythm sees cortisol levels rising to a maximum near awakening and then nearly reaching their minimum by bedtime. Just as resting and reactive heart rate are distinctive components of cardio-vascular activity, baseline and reactive cortisol are distinctive components of HPA axis activity.

Measures of Variation in Baseline Cortisol.

Other types of cortisol measures look at variation in cortisol secretion over the day. The diurnal decline is the difference between maximum levels, which tend to be reached shortly after waking, and minimum levels, which tend to be almost reached by bedtime. The AM-PM ratio is the ratio of summed morning measurements to summed evening measurements. Some researchers also look at the slope of the cortisol secretion curve between various points and use this as a measure of variation in daily cortisol secretion. It has been suggested that lower than average levels of cortisol variation over the day reflect a reduced sensitization of the HPA axis to circadian rhythms and that this may reflect a vulnerability to developing psychosocial problems (Susman et al., 2007).

The Cortisol Awakening Response.

Finally, a distinct component of the daily cortisol rhythm has been identified in which cortisol levels abruptly rise after awakening to reach a daily peak around 20-45 minutes after awakening before gradually declining over the course of the day. This period is known as the cortisol awakening response (CAR). It is considered a genuine response to awakening in that it is dependent upon awakening and not simply a continuation of the pre-awakening circadian rise in cortisol levels (Wilhelm, Born,

Kudielka, Schlotz, & Würst, 2007; Hellhammer, Würst, & Kudielka, 2009). It can be detected in about 75% of healthy subjects and shows a reasonably high stability over time (Würst, Federenko, Hellhammer, & Kirschbaum, 2000). During this period, cortisol levels can increase by up to 150% in adults; however, the increase is lower in children (Rosmalen et al., 2005), suggesting a maturational component to the CAR (Susman et al., 2007). Twin studies have found that it has genetic influences distinct from the heritability of typical baseline levels (Würst et al. 2000; Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma 2003; Kupper et al., 2005). It has also been shown to be independent of the typical baseline levels (Edwards, Clow, Evans, & Hucklebridge, 2001) onto which it is super-imposed. Its pattern of association with health and psychological variables also suggests that it may be a distinctive indicator of HPA axis functioning (Chida, & Steptoe, 2009). Additionally it has been shown to be affected by light levels whereas cortisol secretion later in the day is not (Scheer, & Buijs, 1999). Thus it is likely also under independent regulatory influence from the rest of the diurnal cycle. The CAR is thought to reflect adaptation to stress (Pruessner et al., 2003) and may thus be an important biological marker of adjustment problems in children (Boyce, & Ellis, 2005)

What Affects Cortisol Secretion?

Chronic primary insomnia and acute sleep deprivation can result in greater evening cortisol levels (Ilias, Vgontzas, Provata, & Mastorakos, 2002; Leproult, Copinschi, Buxton, & Van Cauter, 1997; Vgontzas et al., 2001). However the CAR seems unaffected by sleep duration (Pruessner et al.,1997). Social rank is known to affect cortisol levels with those at the bottom of the hierarchy typically showing the

highest levels of cortisol, especially when they are frequently harassed by dominants and have few coping outlets (Abbott et al., 2003). General life stresses and job stress, are also found to affect both baseline cortisol levels and the CAR (for a review, see Chida & Steptoe, 2009). HPA axis dysregulation, especially cortisol hyposecretion, is often observed in children who were neglected and abused (for a review see Golier, & Yehuda, 1998), phenomena that are often associated with chronic negative affect (Egeland, 1991) and conduct problems (Widom, 1989; Lang et al., 2002). Some authors (de Kloet, 2003; Sapolsky, Romero, & Munck, 2000) hypothesize that exposure to chronic or occasional but intense stress can result in down-regulation of the HPA axis, which may explain the findings of cortisol hyposecretion in children who were maltreated.

What are the effects of Glucocorticoid Secretion?

Physiological Effects.

Known long-term (allostatic) effects of the hypersecretion of GCs include a build-up of fat around the midsection (Keltikangas-Järvinen, Räikkönen, Hautanen, & Adlercreutz, 1996), reduced immune system functioning (Wiegers, Croiset, Reul, Holsboer, & de Kloet, 1993), a disruption of learning, memory and synaptic plasticity (McEwen, & Sapolsky, 1995), atrophy of the hippocampus (Sapolsky, 2000), and serious medical conditions such as such as diabetes, hypertension, cancer, and cardiovascular disease (McEwen, 1998; 1998a). Hyposecretion of cortisol also reduces immune system functioning (Wiegers et al., 1993) and impairs memory (Lupien, Gillin, & Hauger, 1999; also see Lupien et al. 2005 for a review), suggesting that optimal physiological functioning requires cortisol levels that are neither too high

or too low, which parallels the inverted U-shaped stress-performance curve mentioned earlier in this chapter. Quadratic relationships between GCs and psychobiological functioning are also observed in the cases of behavioral reactivity towards stimuli in mice (de Kloet, Oitzl, & Joels, 1999) and cognitive performance (for a review see Lupien et al., 2005). Supporting the notion that extreme hypo- or hypersecretion of cortisol is harmful to health, two diseases have been identified, Addison's disease and Cushing's syndrome, which result from hypo- and hyper-secretion of cortisol, respectively (Herbert et al., 2006). Common symptoms of Addison's disease, which can be fatal unlesstreated, include weakness, malaise, weight loss and dehydration with associated low blood pressure while common symptoms of Cushing's syndrome include obesity, muscle weakness, hypertension, hyperglycaemia, decreased immune competence, depression and cognitive impairment (Herbert et al., 2006).

Psychological Correlates.

HPA-axis activation is thought to reflect arousal (Goldsmith, Pellmar, Kleinman, & Bunnay, 2002). However, whereas sympathetic nervous system release of adrenaline and noradrenaline can be felt consciously through associated phenomena such as time distortion (Hancock, & Weaver, 2005) we do not consciously 'feel cortisol', and many of the psychological effects of GCs have yet to be fully elucidated. For instance, while the link between cortisol and depression has been extensively studied, investigators have drawn incomplete, complex and sometimes contradictory conclusions (for a review, see Burke, Davis, Otte, & Mohr, 2005). Burke and colleagues conducted a meta-analysis of the cortisol and depression literature and

concluded that the main finding to be drawn from the literature is that while there is significant individual variation, people with major depressive disorder, as compared with non-depressed individuals, tend to display higher levels of cortisol in the afternoon. Additionally, Stetler & Miller (2005) reported that a blunted CAR was associated with mild to moderate depression in a population sample of women not taking anti-depressants. The CAR has been associated with dysregulated affect in other studies as well (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005; Ellenbogen, Hodgins, Walker, Couture, & Adam, 2006; Steptoe, Gibson, Hamer, & Wardle, 2007; Stetler, & Miller) though the findings are inconsistent (see Chida & Steptoe, 2009 for a review). Additionally, anxiety (Feder et al., 2004; Coplan et al., 2002; Kallen et al., 2008) and various measures of social functioning, such as conduct problems (see next chapter) are associated with HPA axis activity, albeit not always consistently.

Finally, males with Asperger syndrome do not appear to show the typical increase in cortisol levels during the CAR although the rest of their diurnal cycle resembles that of matched controls (Brosnan, Turner-Cobb, Munro-Naan, & Jessop, 2009). Brosnan and colleagues suggest that since autistic children have extreme difficulty coping with change, they are missing a mechanism to adapt to environmental stress. Since the CAR is thought to reflect adaptation to stress (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) an absent CAR may signal that an individual is incapable of properly adapting to his environment.

When the above theory is combined with the notion that early chronic or acute stress can result in later hyposecretion of cortisol (de Kloet, 2003; Sapolsky et al., 2000), a mechanism whereby an adverse childhood can lead to later developmental

problems is revealed. Child abuse, neglect and other trauma may generate an adaptive response aimed at reducing reactivity to the environment. This may translate into a reduced CAR through down-regulation, since the CAR is thought to reflect adaptation to environmental stress (Pruessner et al., 2003). Reducing reactivity to the environment may protect the child from cortisol-related allostatic load (perhaps resulting in the stunting of growth among other things; see Fernald, & Grantham-McGregor, 1998) resulting from probable future adverse conditions. However, it may also handicap the child's ability for future adaptive changes, such as normal processes of socialization, thereby resulting in a failure to desist from trajectories of high levels of conduct problems.

Summary

Hans Seyle described the stress response as a process that allows the organism to cope with an external demand (the stressor) and then to restore homeostasis. When the stressor is not dealt with successfully and homeostasis is not restored, a build-up in stress-response-mediating chemicals effects long-term changes to the organism known as allostasis. A major part of the stress regulating system is the HPA axis, whose end-product in humans is the hormone cortisol. The HPA axis reacts predominantly to stressors that are unpredictable, uncontrollable and long-lasting. In addition to the stress response, other components of HPA axis activity include the baseline diurnal rhythm and measures of its daily variations such as the diurnal decline, measures of slope, the A.M.– P.M. ratio and the CAR, which is a distinct component, likely under different regulatory control and thought to distinctly

represent adaption to stress. The CAR may thus be a good marker for adjustment problems in children.

Chapter 3: Literature Review

Overview

This chapter reviews some of the relevant literature to give context to this study's main contribution: that negative affect mediates the cortisol awakening response (CAR) – conduct problems relationship. Additionally, this review will address reports of gender differences in these relationships so that the reader can appreciate the gender differences found in this study. First, hypothalamic-pituitary-adrenal (HPA) axis – conduct problems findings are covered, followed by possible explanations for the inconsistencies found in this literature. Subsequently, findings of gender differences in these relationships are reviewed. Second, the inconsistent literature on HPA axis – negative affect relationships is summarized and possible ways to reconcile the seemingly disparate findings are presented. Third, negative affect – conduct problems associations are examined. Finally, proposed mechanisms relating HPA axis activity to both conduct problems and negative affect are discussed.

The HPA Axis and Conduct Problems

While antisocial behaviour typically has multiple causes at multiple levels of description, stress-regulating mechanisms appear to be one important component (Kerr, Pagani, Tremblay, & Vitaro, 1997; McBurnett, Lahey, Rathouz & Loeber, 2000; Mezzacappa et al., 1997; Vanyukov et al., 1993). In animal studies, long-term exposure to stress has been shown to generate a growth response in the amygdala which in turn leads to increased levels of aggression (McEwen, 2004). In a study with

rats, it was shown that aggressive behaviour induces corticosteroid (a hormone that is to rats what cortisol is to humans) release but also that injection of a corticosteroid induces aggressive behaviour (Kruk, Halasz, Meelis, & Haller, 2004), demonstrating bidirectional effects. Indeed, hormones may be considered potential causes, consequences, and mediators of conduct problems. For instance, behavioural experiences and sociocultural context lead to hormonal changes that, in turn, influence the expression of conduct problems (for a review see Ramirez, 2003).

In humans, the stress hormone cortisol has been related to individual differences in conduct problems in numerous studies (van Bokhoven et al., 2005; Shoal, Giancola & Kirillova, 2003; McBurnett et al., 2000, 2005; van Goozen et al., 1998; Vanyukov et al., 1993; Pajer, Gardner, Rubin, Perel, & Neal, 2001; van de Wiel, van Goozen, Matthys, Snoek, & van Engeland, 2004; Shirtcliff, Granger, Booth, & Johnson, 2005; Smider et al., 2002; Yu, & Shi, 2009; Dorn et al., 2009; Soderstrom et al., 2004; Gerra et al., 1997; Susman, Dorn, Inoff-Germain, Nottelmann, & Chrousos, 1997; Popma, Doreleijers, et al. 2007; Sodeijker et al., 2007; Fairchild et al., 2008; Popma et al., 2006; Snoek, van Goozen, Matthys, Buitelaar, & van Engeland, 2004; van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000). However, the relationship between cortisol and conduct problems appears to be complex and the above studies have yielded contradictory results. A first set of findings comes mainly from male and clinical samples and has shown that low baseline levels of cortisol are associated with high levels of conduct problems (McBurnett et al., 2000; Vanyukov et al.; Pajer et al.; van de Wiel et al.; Shirtcliff et al.; Smider et al.; Yu, & Shi; Dorn et al.; Popma, Doreleijers, et al., 2007). In addition, a population-based, five-year longitudinal study found that low

morning cortisol levels predicted later conduct problems in adolescent boys (Shoal et al.). Others studies have reported that conduct problems are associated with low cortisol reactivity (Fairchild et al.; Popma et al. 2006; Snoek et al., 2004; van Goozen et al., 1998/2000; McBurnett et al. 2005). Another set of findings indicates that conduct disorder (CD) in boys and male adolescents is associated with elevated levels of morning cortisol (van Bokhoven et al. and Fairchild et al., respectively), evening cortisol (Fairchild et al.), that high cortisol reactivity in men is associated with high levels of physical aggression in an induced aggression task (Gerra et al.) and that high cortisol reactivity predicted elevated levels of conduct problems in adolescents one year later (Susman et al.). Violent offenders have also been found to have significantly higher cortisol levels as compared to healthy controls (Soderstrom et al.). Finally, there are also many studies which did not find any relationship between cortisol and conduct problems (Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Kruesi, Schmidt, Donnelly, Hibbs, & Hamburger, 1989; Scerbo, & Kolko, 1994; Schultz, Halperin, Newcorn, Sharma, & Gabriel, 1997; Stoff et al., 1992; Susman et al., 1999).

Possible Explanations for the Inconsistencies

Methodological Explanations.

There are many possible ways of resolving the above-mentioned inconsistencies. For instance, Popma, Doreleijers, et al. (2007) suggested that methodological issues relating to the measurement of cortisol may be at least partially responsible for the inconsistency and it is true that very few of the above studies would be judged methodologically sound according to the stringent criteria of Lupien

et al. (2006) and Goodyer et al. (2001; see the next chapter for more details). Although a careful examination of the methodology of all the studies above is beyond the scope of this review (however some further examination can be found in the next chapter), some key points are addressed below.

Only five of the studies mentioned above reported a positive relationship between cortisol levels and conduct problems while sixteen reported negative association and six reported no association. It is possible that those five positive association studies can be dismissed on various grounds. For instance, the Soderstrom et al. (2004) study is plagued by multiple methodological issues which drastically reduce the strength of their results. First, although it claims to be a study of violent offenders, it is actually a study of *suspected* violent offenders awaiting trial, thus not all of the supposed violent offenders may actually be violent or offenders. Second, it attempts to measure baseline morning cortisol levels in blood and cerebrospinal fluid, which requires invasive sampling procedures likely to induce a reactive cortisol response (Gröschl & Rauh, 2006; Granger et al., 2007) thus confounding their measures. Third, because these individuals are undergoing psychiatric evaluation prior to their court trials, this is likely a very stressful time for them and therefore their cortisol levels may be elevated above their normal levels. Fourth, the control group was a convenience sample of medical students not matched for age or sex despite the fact that age and sex differences in cortisol measures have been reported (e. g. Rosmalen et al., 2005; Dorn et al., 2009; Marsman et al., 2008). Interestingly, among the suspected violent offenders diagnosed with conduct disorder or antisocial personality disorder, lower levels of cortisol were observed, although this group was

too small to permit meaningful statistical comparisons. Because of all of these problems, this study can easily be dismissed on methodological grounds.

The van Bokhoven et al. (2005) study also suffers from one key methodological issue: the authors were attempting to measure early morning baseline cortisol, sampled as participants arrived at the laboratory. However, there was a greater than one hour time difference between the earliest sampling and the latest sampling. Because the period after awakening is characterized by rapid change in cortisol levels, without knowing how much time has elapsed since awakening, cortisol measures obtained during this time period are highly unreliable. Indeed, Popma, Doreleijers, et al. (2007) suggest that not taking into account time of awakening may be responsible for the inconsistencies in this literature. Additionally while van Bokhoven and colleagues did find a small but significant negative association between time of sampling and cortisol concentration, there was no control for this or time of awakening (which was not recorded) in the analyses. Therefore conclusions from this study rest on a weak methodological foundation.

Finally, Fairchild et al. (2008) note that their finding that adolescents with CD (who sampled their own saliva at home) had higher levels of evening cortisol may have been due to these participants' non-compliance with experimental protocols. Non-compliance is a characteristic of individuals with conduct problems (see Chamberlain & Patterson, 1995; Loeber et al., 1993; McMahon & Forehand, 2003). Thus correct timing and compliance with other experimental protocols (such as not smoking or taking drugs within two hours of sampling) were unlikely to have been achieved since these adolescents demonstrated irregularity in self-reported timing as compared with controls and nearly three quarters reported smoking tobacco while

nearly half reported smoking marijuana regularly. However, the authors did find small elevations in CD participants' 11 AM cortisol levels as compared to normal controls when sampling under laboratory conditions (note that they did not record or report time of awakening for this day). A problem with this finding is that on days other than their laboratory visit, these adolescents had typically reported waking up about one and half hours later than controls; so their 11 AM measurement would be analogous to a 9:30 AM measurement for controls, and thus phase differences in their sleep-wake cycles could easily account for the small difference in cortisol observed.

The two remaining positive association studies (Gerra et al., 1997; Susman et al., 1997) measured reactive and not baseline cortisol, which is a distinct component of HPA axis activity. It is possible that baseline cortisol is negatively and reactive cortisol positively associated with conduct problems. Thus these two studies may not in fact be inconsistent with the vast majority of the literature reporting negative baseline cortisol – conduct problems associations but may complement it by showing a different association with a different measure of HPA axis functioning. However, others have reported lower cortisol reactivity in individuals with conduct problems (Fairchild et al., 2008, Popma et al., 2006; Snoek et al., 2004; van Goozen et al., 1998; van Goozen et al., 2000; McBurnett et al., 2005) thus calling the above two results into question.

Proactive vs. Reactive Aggression Hypothesis.

In order to explain the last inconsistency, McBurnett et al. (2005) suggested that since positive reactive cortisol – conduct problems associations were reported by studies using population-based samples whereas negative reactive cortisol – conduct

problems associations were reported mainly by studies using clinical samples, the inconsistency may be due to differences between clinical and non-clinical populations. They go on to speculate that reactively aggressive individuals with conduct problems are probably more common in the population-based studies whereas proactively aggressive, psychopathic individuals with CD are probably more common in clinical samples. Thus it could be possible that the reactively aggressive participants more likely to be found in population-based samples are characterized by higher cortisol reactivity whereas the proactively aggressive participants more likely to be found in the clinical samples are characterized by lower cortisol reactivity. This explanation is plausible and requires further investigation.

Inverted U-Shaped Hypothesis.

Another possible way to resolve the apparent inconsistency in both the baseline and reactive literature is to suppose that both negative and positive relationships are possible. For instance, some propose (Hebb, 1955) that both high and low levels of arousal represent aversive states (such as negative affect) and others (Anderson, & Bushman, 2002) propose that aversive states can lead to the expression of conduct problems. HPA-axis activity is thought to reflect arousal (Goldsmith et al., 2002); thus, to resolve the literature's inconsistent cortisol-conduct problems findings, Pihl, Vant, and Assaad (2003) proposed a hypothesis involving a U-shaped relationship between arousal (on the X axis) and conduct problems (on the Y axis) in which such problems are more likely to occur when cortisol levels are high or low than when cortisol levels are intermediate. This is consistent with Lupien et al.'s (2006) hypothesis of an inverted U-shaped relationship between cortisol and

psychosocial functioning in general. Such a relationship would not be surprising because U-shaped relationships between glucocorticoids (GCs; see Chapter II) and psychobiological functioning are commonly observed, such as in the cases of working memory (Lupien, Gillin & Hauger, 1999), immune functioning (Wiegers et al., 1993), behavioural reactivity towards stimuli in mice (de Kloet, Oitzl, & Joels, 1999), hippocampal long-term potentiation and cognitive performance (Lupien et al. 2005).

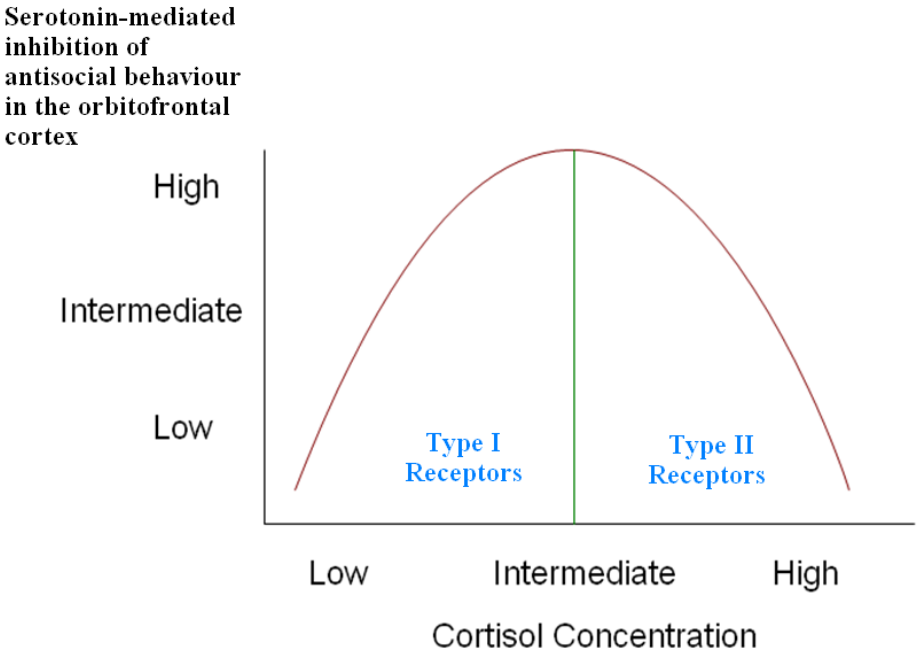
Neurophysiological Model.

To explain some of the quadratic GC - psychobiological functioning relationships, de Kloet (2003) proposed a neurophysiological model in which the two subtypes of GC receptors, type I which have priority in binding with GCs and type II which bind to the left-over GCs, have opposite effects. This model predicts U-shaped relationships because the effect of type I receptors, which become activated as cortisol levels increase from low to intermediate, is counteracted by the effect of type II receptors, which become activated as cortisol levels increase from intermediate to high. In fact these two receptor subtypes have already been shown to have opposite effects on serotonin-induced inhibition in the hippocampus and dentate gyrus (for a review see de Kloet, 2003). Because serotonergic activity in the orbitofrontal cortex, which contains both type I and II receptors (Lupien et al., 2006), likely plays a crucial role in inhibiting antisocial behaviour (Davidson, Putnam, & Larson, 2000), it is possible that in this region of the brain, activation of type I receptors increases and activation of type II receptors decreases serotonin-induced inhibition of antisocial behaviour, resulting in a U-shaped relationship between cortisol levels and conduct

problems. This last hypothesis is an original one, conceived by the author of this thesis. Figure 1 below illustrates this hypothesized relationship. This model is consistent with Shoal et al.'s (2003) finding that the primary personality mediator between low cortisol and aggression was a lack of self-control, or inhibition.

Figure 1

Neurophysiological model of the relationship between cortisol and antisocial behaviour



More methodological issues.

While the above model is interesting, the dismissal of the three studies finding positive relationships between supposedly baseline cortisol measures and conduct problems (Soderstrom et al., 2004; van Bokhoven et al., 2005; Fairchild et al., 2008) on methodological grounds is a simpler explanation, requiring fewer additional hypotheses. Thus perhaps baseline cortisol is in fact negatively related to conduct problems, in accordance with the majority of studies. However, the majority of

studies are also plagued by methodological problems involving the measurement of baseline cortisol (described in more detail in the next chapter); thus their results should be interpreted cautiously. Despite this, the fact that the negative association between cortisol and conduct problems has been replicated numerous times suggests that this is indeed the true nature of the relationship, at least for boys.

Gender Differences in Cortisol – Conduct Problems Relationships

Gender differences in the expression of conduct problems are widely reported (for instance, see Archer, 2004 for a review of sex differences in aggression) and it seems plausible to expect that psychoneuroendocrine factors play a role. HPA axis activity is reported to be differently related to a wide variety of psychosocial factors in males and females such as attention problems (Susman et al., 2007), sensation-seeking (Rosenblitt, Soler, Johnson, & Quadagno, 2001), social cognition under stress (Smeets, Dziobeck, & Wolf, 2009), decision making after a stressful task (van den Bos, Harteveld, & Stoop, in press), callous-unemotional traits (Loney, Lima, & Butler, 2006), and violence exposure in urban youth (Kliewer, 2006). A few studies have also found gender differences in the cortisol – conduct problems relationship (Dorn et al., 2009; Sondeijker et al., 2007; Shirtcliff et al., 2005; Smider et al., 2002). For instance, in a fairly methodologically sound study of children with disruptive behaviour disorders (DBD; includes oppositional defiant disorder and CD), it was found that DBD boys and healthy control girls had the lowest levels of baseline cortisol (Dorn et al., 2009). Unfortunately, only 39 out of 180 participants were girls; so the gender difference result must be interpreted with caution. However, another study found a similar effect in that girls had a positive while boys had a negative

relationship between baseline cortisol levels and conduct problems (Sondeijker et al., 2007). Additionally, it has been reported that the cortisol – conduct problem relationship is present in boys but not in girls (Shirtcliff et al., 2005; Smider et al., 2002). Many studies did not report gender differences but most studies in this field exclusively looked at boys or included so few girls that such differences would be difficult to detect. Thus it is likely that cortisol – conduct problems relationships are either weaker in girls, absent entirely or opposite in direction to that of boys.

To explain such gender differences in hormone - behaviour relationships, several moderating influences, both social and biological are possible. For instance, it has been proposed that the differential socialization of boys and girls may be responsible (Rosenblitt et al., 2001). Biological influences on behaviour may be magnified in boys who are socialized to 'express' and attenuated in girls who are socialized to 'suppress' (for reviews see Block, 1983; Rury, 1987; Bussey & Bandura, 1999; Udry, 2000; Zelezny, Chua, & Aldrich, 2000). Additionally, Sondeijker et al. (2007) postulated that estrogens, which are known to affect HPA axis activity (Burgess, & Handa, 1992; Handa, Burgess, Kerr, & O'Keefe, 1994; Roy, Reed, & Van Vugt, 1999; Vamvakopoulos, & Chrousos, 1993), may be responsible for the gender differences observed; however they note that such a hypothesis was purely speculative and did not specify the mechanism by which estrogen could influence the expression of conduct problems. Interestingly, the findings of the study featured in this thesis may help to elaborate Sondeijker et al.'s hypothesis by suggesting just such a mechanism. The main finding of the study featured in this thesis is that negative affect mediates the cortisol – conduct problems relationship. As such, estrogens, which have a positive effect on mood (as assessed by hormone

replacement therapy studies; see Pearce, Hawton, & Blake, 1995; Klaiber, Broverman, Vogel, & Kobayashi, 1979; Sherwin, 1991) may be differentially protecting girls from the effects of morning cortisol on negative affect. Indeed it was found by the featured study that the CAR – negative affect relationship was stronger for boys than for girls, as was also found by Polk et al. (2005). Finally, as a consequence of the findings of the study featured in this thesis, an original biological explanation is proposed in the last chapter. In short, it is hypothesized that pre- and peri-natal testosterone exposure preferentially sensitizes boys (because they are exposed to more testosterone during this period, which results in their masculinization and defeminization) to the effects of low cortisol on negative affect, which in turn contributes to their conduct problems.

The HPA axis and Negative Affect

Many studies have associated HPA axis activity with individual differences in negative affect and depressive symptoms (Polk et al., 2005; Ellenbogen et al., 2006; Steptoe et al., 2007; Stetler, & Miller, 2005; Fortunato, Dribin, Granger, & Buss, 2008; Hanson, Maas, Meijman, & Godaert, 2002; also see Burke et al., 2005 for a meta-analysis). For instance, Fortunato and colleagues measured morning cortisol levels in toddlers and found them to be positively associated with negative affect during various laboratory tasks designed to elicit emotions and behaviour. The Hanson group found baseline cortisol throughout the day to be positively associated with negative affect in adults. Somewhat similarly, a meta-analysis of the cortisol and depression literature concluded that the main finding to be drawn from the literature is that while there is significant individual variation, people with major depressive

disorder, as compared with non-depressed individuals, tend to display higher levels of cortisol in the afternoon (Burke et al., 2005). However most studies do not control for time of awakening, which reduces the reliability of their results (see chapter IV for a detailed explanation). Thus if the sleep-wake cycle of depressed people and/or those with negative affect were significantly phase-shifted forward as compared to people without depression or high negative affect, then that might account for their higher levels of cortisol, since they have had less time since awakening for their cortisol levels to decline. Nevertheless, it seems likely that baseline cortisol levels, especially when measured later in the day, are positively related to negative affect and depressive symptoms. It is also possible that as the day progresses, negative and depressive people may be more likely than non-negative and non-depressive people to experience stress that elevates their cortisol levels. This may be due to their negatively-biased social and affective information processing (Gollan, Pane, McCloskey, & Coccaro, 2007), and potentially greater exposure or vulnerability to the stress of daily hassles (see Sher, 2004).

The CAR and Negative Affect.

The CAR is of particular interest when studying the relationship between HPA-axis activity and negative affect because recent studies have associated it with negative affect but have failed to find associations with underlying baseline waking levels (Polk et al. 2005; Ellenbogen et al. 2006; Steptoe et al. 2007; Stetler and Miller, 2005). This suggests that previous associations between morning cortisol levels and negative affect (Fortunato et al., 2008) may actually been due to differences in the CAR. However, there are many contradictory findings linking the

CAR to emotional states (for a review see Chida & Steptoe, 2009). Polk et al. found that the CAR is positively associated with negative affect in men but not in women and Steptoe et al. found that positive affect measured by brief self-reported ratings at multiple pre-determined time points, but not by questionnaire, was negatively associated with the CAR in a sample of men but found no relation with negative affect. On the other hand, Ellenbogen et al. reported that cortisol levels 60 minutes after awakening (a measure related to the CAR) are positively associated with positive affect in adolescents and Stetler and Miller reported that the CAR was negatively associated with mild to moderate depression in a population sample of women not taking anti-depressants.

Methodological issues may contribute to the inconsistencies. For instance, the studies associating a low CAR with either reduced positive affect or increased negative affect generally employed more days of measurement and better CAR sampling procedures [2 days of measurement and written confirmation of sampling time in both the Ellenbogen et al. (2006) and the featured study of this thesis; three days of measurement and compliance monitoring in the Stetler and Miller, (2005) study] than studies associating a low CAR with reduced negative affect [one day of measurement in a hotel during a potentially stressful virus challenge study in the Polk et al. 2005 study, and two days of measurement and written confirmation of sampling time in the Steptoe et al. 2007 study]. It is also possible that the inconsistent results can be reconciled by considering the possible moderating roles of age and sex, since reduced positive or increased negative affect is associated with a low CAR in male children (the study featured in this thesis) adolescents of both sexes (Ellenbogen et

al.) and adult women (Stetler & Miller) whereas reduced negative affect is associated with a low CAR only in adult men (Polk et al.; Steptoe et al.).

The above explanations make the questionable assumption that positive and negative affect are opposites and may not be found together. Indeed, controversy surrounds this issue with some assuming that they are opposites while others maintain that they are independent (Feldman Barrett, & Russell, 1999). While none of the above-mentioned studies examined this, Polk et al.'s (2005) pattern of results suggested that positive and negative affect had opposite directions of association with cortisol measures while Ellenbogen et al. (2006) found similar directions of association between positive and negative affect on the one hand and cortisol measures on the other (though not all of these associations were significant).

Finally, to tie this in with the general HPA axis – negative affect literature, it may be possible that CAR is negatively associated with negative affect while baseline cortisol levels later in the day are positively associated with negative affect. Different mechanisms may be involved. For instance, since the CAR may reflect ability to adapt to the environment (Brosnan et al., 2009), perhaps individuals with an attenuated CAR may have trouble 'fitting in' and may experience negative affect as a result. This may lead to greater social stress throughout the day, causing their afternoon and evening levels of cortisol to be elevated relative to more adaptable individuals.

Negative Affect and Conduct Problems

Numerous studies have reported positive associations between negative affect and conduct problems (Belsky, 2004; Belsky, Hsieh, & Crnic, 1998; Loney et al.,

2006; Séguin, Arseneault, Boulerice, Harden, & Tremblay, 2002; Schmitz et al., 1999; Gjone, & Stevenson, 1997). Additionally, McBurnett et al. (2005) subjected male children and adolescents to two different types of stressors and found resulting negative affect to be positively and resulting positive affect to be negatively associated with conduct problems. Somewhat similarly, Van Goozen et al. (2000) found that DBD children had higher negative affect under stress than normal controls. Interestingly, both these studies also reported that children with conduct problems had lower cortisol reactivity under stress than normal controls.

Theories of HPA axis Relationships with Conduct Problems and Negative Affect

Van Goozen et al. (2000) suggested that low HPA axis activity may be a marker for chronic and/or acute childhood stress such as abuse or neglect (for a review see Golier, & Yehuda, 1998) and abused or neglected children may develop hostile-world cognitive schemas, as a consequence of their maltreatment, biasing them towards negative interpretations of social situations which may in turn contribute to their conduct problems (Dodge, Pettit, Bates, & Valente, 1995; Weiss, Dodge, Bates, & Pettit, 1992). Additionally, HPA axis activity may reflect individual differences in physiological arousal (Goldsmith et al., 2002), which plays a role in theories of the development and expression of conduct problems such as fearlessness theory (Raine, 1993) and sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979), the latter of which sees low arousal as an aversive state, in line with Hebb (1955). Additionally, aversive states such as negative affect may contribute to conduct problems via other mechanisms such as by increasing irritability and frustration, for instance (see Berkowitz, 1980; Berkowitz, 1993). These theories may

be complementary and it is possible that both fearlessness and sensation-seeking as well as other mechanisms, such as negative affect, mediate various HPA axis – conduct problems relationships. Finally, negative affect as a proposed mediator of the HPA axis – conduct problems relationship is concordant with Susman et al. (1987)'s hypothesis that emotions may be the mechanism by which the effects hormones are exhibited in anti-social behaviour.

Summary

In humans, the stress hormone cortisol has been associated, usually negatively, with individual differences in conduct problems in numerous studies, although the literature is plagued with inconsistencies and methodological problems. Methodological considerations, as well as quadratic cortisol – conduct problems relationships and differences in aggression subtypes between populations studied, have been advanced as explanations of the inconsistency. Sex differences are commonly observed with cortisol levels more negatively associated with conduct problems in boys than in girls. Possible explanations of the sex differences include socialization differences in which boys are taught to express and girls to suppress as well as interactions with gonadal hormones The negative affect - cortisol literature is highly inconsistent; however the trend is towards positive relationships between negative affect and evening measures of cortisol. The CAR appears to be more related to negative affect than underlying waking levels; however, the reported directions of association are inconsistent. Negative relationships between negative affect and the CAR are typically reported in the studies with superior methodology and this is highly consequential as measurement of the CAR is especially unreliable

when methodology is not strong. Individuals with conduct problems tend to be high in negative affect, yet their emotions seem dissociated with their cortisol reactivity, which tends to be lower than individuals without conduct problems. Theories that may explain the low cortisol – conduct problems findings include fearlessness theory and sensation seeking theory. Finally, it has been proposed that emotions may be the mechanism by which the effects hormones are exhibited in antisocial behaviour. This is concordant with the main finding of the research featured in this thesis, that negative affect mediates the CAR – conduct problems relationship in boys.

Chapter 4: Methodology

Overview

This relatively short chapter discusses important methodological issues related to the measurement of conduct problems and cortisol. It serves to give a methodological background to the readers so that they can appreciate the featured study's methodology and limitations, which are discussed in subsequent chapters. First, teacher, parent and self-report methods for assessing conduct problems are compared. Second, different types of instruments for measuring conduct problems are described. Third, issues and recommendations relating to measuring baseline cortisol are presented. Finally, the lengthiest section explains the advantages and discusses some challenges related to the measurement of the cortisol awakening response (CAR). This chapter concludes with a paragraph of recommendations for future studies.

Measuring Conduct Problems

Measuring conduct problems such as aggressive behaviour is a difficult task and this difficulty stems directly from how modern researchers define aggression. Buss (1961) defined aggression as 'an attempt to cause harm'. This definition, along with the occasional minor variation, is how most researchers define aggression today. Thus, categorizing an act as aggression is dependent upon an observer inferring 'intent to harm' on the part of the perpetrator. This may be difficult as even sports researchers and game officials often have trouble judging whether a player intended to harm or not (Mattesi, 2002).

Issues Regarding Types of Informants for Assessing Conduct Problems.

In studies of conduct problems in youth, parents or teachers are often the informants although child self-report is sometimes used. Only modest associations are usually reported between parent and teacher ratings of aggression, suggesting that inter-informant reliability of some such measures may be low. On the other hand, self-report is plagued by various problems (especially in young children; see Boyle et al., 1993; Edelbrock, Costello, Dulcan, Kalas, & Conover, 1985; Schwab-Stone, Fallon, Briggs, & Crowther, 1994), such as social desirability issues, and potentially confounded by non-compliant and deceitful traits in individuals with conduct problems. While certain interview-based self-report instruments, such as the Dominic-R and the Berkley Puppet interview, have shown moderate external validity in 5 and 7 year olds (Arseneault, Kim-Cohen, Taylor, Caspi, & Moffitt, 2005), they can be relatively costly and time consuming. Thus self-report is probably the least desirable method for obtaining valid and reliable estimates of conduct problems in children.

It is difficult to say whether parent or teacher reports are superior and it is possible that the expression of conduct problems may be moderated by context. Thus parent and teacher reports may be measuring different things. For instance, a child who is harshly physically disciplined and/or tightly controlled by parents may behave well at home but act out in school. Conversely, a child who is ignored by parents may act up at home to get attention but may behave well in school. Additionally, a child who is non-compliant by nature may not display conduct problems in a home with lax parental discipline but may rebel in a comparatively authoritarian school environment. Finally, parents and teachers may observe different levels of conduct

problems because of other facilitating or inhibiting environmental factors such as the presence of deviant peers and hostile siblings. Therefore, including both parent and teacher reports of conduct problems would potentially allow conduct problems to be more broadly assessed. Unfortunately for the present study, teachers in Montreal, Quebec, Canada were on strike during the collection phase and thus did not complete questionnaires.

Instruments used to Assess Conduct Problems.

Many different instruments are used to assess conduct problems such as the Achenbach family of instruments which includes the empirically constructed Child Behavior Checklist (CBCL; Achenbach, 1991a) for the mothers and the Teacher's Report Form (TRF; Achenbach, 1991b) for the teachers. These instruments ask informants to answer each item as being either *not true* (0), *somewhat* or *sometimes true* (1), or *very true* or *often true* (2) in response to a statement about the child's behaviour. This set of instruments is primarily used in the United States although it is also used elsewhere. It has been shown to possess both reliability and validity (for a review see Lowe, 1998). Another way of assessing conduct problems, especially common in the United States, is to measure DSM-IV-TR (American Psychiatric Association, 2000) symptoms of oppositional defiant disorder and conduct disorder using, for instance, the Adolescent Symptom Inventory-4 (Gadow, & Sprafkin, 1998). It is a parent checklist measure used commonly in both research and clinical settings and has demonstrated good evidence of internal consistency, test–retest reliability, and convergent validity (see Gadow, & Sprafkin, 1998). In Canada, conduct problems are sometimes assessed with the behaviour measures that were

collated for the National Longitudinal Survey of Children and Youth (Statistics Canada, 1996), from a variety of sources, by a panel of experts.

Measuring Cortisol Levels

Most of the studies on cortisol and conduct problems attempted to measure baseline cortisol, which is someone's average daily level of cortisol in the absence of any unusual stress. This contrasts with another type of cortisol measurement: reactive cortisol, which measures the change in cortisol levels from before to after the introduction of a stressor. While measuring reactive cortisol is as easy as taking the difference between pre and 17-32 minute-post-stress samples (see Stansbury & Gunnar, 1994 and Gunnar & White, 2001), obtaining an accurate measurement of baseline cortisol is surprisingly difficult and unlikely to have been achieved in many previous studies in this field. This may underlie inconsistencies in the literature examining baseline cortisol and conduct problems (Popma, Doreleijers, et al., 2007).

Measuring Baseline Cortisol.

Cortisol secretion follows a diurnal rhythm with a 10-fold variation in concentration from 8 A.M. to 8 P.M. in the saliva of 12-18 year olds (Goodyer et al. 2001). Since cortisol concentration is highest upon waking and declines for the rest of the day, two people with the same baseline cortisol levels may have very different cortisol scores, even if measured at the same time of day, if one person woke up before the other. It is difficult to know what exactly a series of baseline cortisol measures means without the context of the individual's daily rhythms and the extent of exposure to, reaction to, or recovery from a stressful experience. For example,

participants in studies typically engage in a variety of tasks during their visit to the lab including cognitive testing, interviewing and answering questionnaires, actions that are known to stimulate the HPA axis (Steptoe, Kunz-Ebrecht, Wright, & Feldman, 2005) thus potentially confounding baseline cortisol measures. Though in some studies the time of cortisol collection did not seem to affect the relationship between cortisol concentration and the dependent measure (McBurnett et al., 2000; Sapolsky, Alberts & Altmann, 1997), the idiosyncrasies of an individual's biological rhythms are not entirely accounted for, especially when time of awakening is not controlled for which unfortunately is the case for the vast majority of studies.

While there is no widely accepted standard for obtaining an accurate measurement of baseline cortisol, some key principles have been identified, which are relevant for future research. Lupien et al. (2006) stress the importance of controlling for the time of day and making several measurements, not just a morning sample. Goodyer et al. (2001) goes so far as to suggest a minimum of four days of measurement. Engaging in physical exercise, smoking, eating, drinking, using caffeine, alcohol, or other drugs and chewing gum near to sampling times are all thought to reduce the reliability of saliva sampling (Kudielka, Broderick, & Kirschbaum, 2003) and thus need to be experimentally controlled for. Many studies (including the one featured in this thesis) also include measures of concurrent stress and affect to see if they are affecting cortisol levels (they did not in this study). Upon examination of the literature, it becomes clear that few of the previous studies followed more than one of the above recommendations and almost none followed the majority of them.

Measuring the Cortisol Awakening Response (CAR).

The CAR is measured in different ways and the distinctions are important for the reliability of the measures and the interpretation of the scores obtained. The total secretion of cortisol, estimated by calculating the area under the curve (AUC; see Pruessner et al., 2003) during the CAR (CAR_{auc}) is popular way of "measuring the CAR" that is correlated with average daily cortisol levels (Edwards et al., 2001). It is a measure that combines underlying average daily levels with the response to awakening. Because it combines two distinct components of HPA axis activity and thus two distinct sources of variation in morning cortisol levels (the CAR and underlying baseline waking levels), it is confusing to interpret, and some have chosen to interpret it in line with average daily levels and not in line with a response to awakening (e.g. Marsman et al., 2008). Thus it is always recommended to measure the actual response to awakening (Pruessner et al., 2003).

A more straightforward and valid way of measuring the CAR is to focus on the relative increase in cortisol levels from the awakening to post-awakening measures. The increase itself (CAR_i) is considered a genuine response to awakening in that it is dependent upon awakening and not simply a continuation of the pre-awakening circadian rise in cortisol levels (Wilhelm et al., 2007; Hellhammer et al., 2009). It has also been shown to be independent of the typical baseline levels onto which it is super-imposed (Edwards et al., 2001). Its pattern of association with health and psychological variables also suggests that it may be a distinctive indicator of HPA axis functioning (for a review and comparison with the CAR_{auc} see Chida and Steptoe, 2009). Finally, while the theoretical significance of the CAR_{auc} is not clear, the CAR_i is thought to represent adaptation to stress (Pruessner et al. 2003) and thus

theoretically, it should be an important biological marker of adjustment problems in children (Boyce & Ellis, 2005).

Measuring the CAR has certain methodological advantages over measuring other components of daily cortisol secretion. Because it takes place right after awakening, there is less opportunity for unexpected stressful events to cause HPA axis reactivity and interfere with the measurements as compared with measurements taken later in the day. Second, because the timing of the measurements are set in relation to the participant's sleep-wake cycle, phase differences between participants' sleep-wake cycles don't affect the validity of the measurement as they might with other measurements of daily cortisol where the measurements are timed in relation to standard time (example: the AM-PM ratio). On the other hand, obtaining an accurate measure of the CAR is highly dependent on the accurate timing of measurements in relation to awakening, more so than other measurements of daily cortisol variation because of the relatively brief time window in which the CAR is observable (Kudielka et al. 2003; Broderick, Arnold, Kudielka, & Kirschbaum,. 2004). Weekend – weekday differences have been reported for the CAR (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Schlotz, Hellhammer, Schulz, & Stone, 2004; Thorn et al., 2006) and some have found the magnitude of the CAR to be related to the anticipated stressfulness of the day (Stalder, Evans, Hucklebridge, & Clow, in press). Thus it is advisable to experimentally control for weekend-weekday effects. Finally, though direct empirical evidence is lacking, it makes sense to measure the CAR on non-consecutive days so as to avoid systematic within subject error as a result of localized (in time) stressful events.

Summary

Parent and teacher reports are generally more effective at detecting children's conduct problems than child self-report, especially in young children. Nevertheless, reports from multiple informants may be useful for detecting context (e.g. at home, at school or when the child is alone)-dependent expression of conduct problems. Non-reactive cortisol sampling must be done on more than one day and always taking into account the individuals' sleep-wake cycle as well as other potential confounds such as concurrent stress. Studies measuring post-awakening cortisol should always measure the CAR (meaning the CAR_i) because it is an independent contributor to post-awakening cortisol levels. Moreover, the CAR is thought to represent adaptation to stress and may thus be of special significance as a marker of psychosocial maladjustment in children. However, measuring the CAR is challenging because of the brief time-window in which it is observed, making accurate timing of measurements especially important. Finally, when measuring the CAR one must also control for weekday-weekend effects which may be due to lower anticipated stress of weekend days as compared with weekdays.

Chapter 5: The Featured Study

Overview

This chapter is comprised of an article in the final phases of preparation for submission to the journal Hormones & Behavior. The article begins with an abstract and is then followed by an introduction which briefly summarizes the cortisol – conduct problems literature and suggests that methodological issues both underlie the inconsistencies in that literature as well as the previous failures to detect a relationship between the cortisol awakening response (CAR) and conduct problems in boys. Subsequently, the hypothesis is put forward that negative affect may mediate the suspected relationship between the CAR and conduct problems. The methods section then describes the sample characteristics, recruitment methods, saliva sampling, behavioural measures, hormonal assaying and data analysis methods. Then, the results section describes how the hypotheses presented in the introduction were supported as well as reporting some peripheral findings. Finally, the discussion serves both as a summary for the article and this chapter as well as a complement to the discussion in the final chapter of this thesis. Included at the end of the chapter is a list of references, three figures referred to in the results section and the declaration concerning the contribution of each author to the research and writing of the article.

Negative affect mediates the relationship between the cortisol awakening response and conduct problems in boys

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Abstract

Hyposecretion of hypothalamic-pituitary-adrenal (HPA) axis hormone cortisol has been associated with conduct problems mostly in boys, and much less often in girls. Further, negative affect has been linked to both conduct problems and the Cortisol Awakening Response (CAR). We hypothesized that negative affect acts as a mediator in cortisol-conduct problems relationships in boys. We tested this hypothesis with 80 boys and 68 girls recruited from a larger population-based longitudinal sample at 10 years of age. Conduct problems and negative affect were assessed by mother-report of child behavior. Cortisol was assayed from saliva samples collected by parents upon the child's awakening and 30 minutes thereafter on two non-consecutive school days and provided an index of the CAR. There was no significant difference in the CAR between boys and girls. However, in boys but not in girls, a reduced CAR was associated with increased negative affect and conduct problems. These sex differences in the correlation coefficients were significant. As predicted by the model, negative affect was found to completely mediate the relationship between boys' CAR and conduct problems. These results suggest that an attenuated CAR is a marker for emotional dysregulation in boys which when manifested as negative affect may lead to conduct problems. This ties in to theories of arousal and conduct problems which propose that low HPA axis activity reflects physiological under-arousal which is experienced as an aversive state and predisposes individuals to display conduct problems.

Key words: Cortisol Awakening Response, Conduct Problems, Negative Affect, Gender Differences, CAR.

All conduct problems share common causal mechanisms to some degree (Lahey and Waldman, 2007) and there is evidence to suggest that children's conduct problems may stem from neurobiological deficits (Raine, 2002). For instance, stress regulating mechanisms, particularly the hypothalamic-pituitary-adrenal (HPA) axis, appear to contribute to individual differences in conduct problems (Kruesi et al. 1989; Raine, 2002; Vanyukov et al. 1993; Kerr et al. 1997; Mezzacappa et al. 1997; McBurnett et al. 2000). The majority of studies linking HPA axis activity to conduct problems have mainly used male participants and have shown that low levels of cortisol, the end product of the HPA axis in humans, are associated with high levels of conduct problems (McBurnett et al. 2000, 2005; Vanyukov et al. 1993; Pajer et al. 2001; van de Wiel et al. 2004; Shirtcliff et al. 2005; Smider et al. 2002; Yu and Shi, 2009; Dorn et al. 2009; Popma et al. 2007; Oosterlaan et al. 2005; Fairchild et al. 2008, Popma et al. 2006; Snoek et al., 2004; van Goozen et al. 1998; van Goozen et al. 2000). In addition, a longitudinal study has found that low levels of cortisol in school age boys predict aggression five years later in mid-adolescence (Shoal et al. 2003). In contrast, several studies have reported a positive relationship between cortisol levels and conduct problems (van Bokhoven et al. 2005; Soderstrom et al. 2004; Gerra et al. 1997; Susman et al. 1997; Fairchild et al., 2008). There are also many studies which did not find any relationship between cortisol levels and conduct problems (Klimes-Dougan et al. 2001; Kruesi et al. 1989; Scerbo and Kolko, 1994; Schultz et al. 1997; Stoff et al., 1992; Susman et al., 1999).

Key methodological issues may underlie some of the inconsistencies reviewed above. Cortisol secretion follows a diurnal rhythm with a 10-fold variation in concentration over the day (Goodyer et al. 2001). Since cortisol concentration is

highest shortly after waking and then declines for the rest of the day, the findings of higher cortisol levels in individuals with conduct problems may have been due to their tendency to wake up significantly later than individuals without conduct problems (Fairchild et al. 2008). Additionally, not controlling for the time of awakening results in an unreliable measure of baseline cortisol and thus should increase the probability of type II error which could explain some negative findings.

Few studies have accounted for individual differences in awakening time, though some exceptions are Popma et al. (2007), who found that young adolescent boys with disruptive behavior disorder had lower levels of cortisol in the first hour after awakening as compared with normal controls, and Sondeijker et al. (2007) who found similar results for boys with conduct problems in a population sample. By measuring cortisol levels during the first hour after awakening, these studies have improved upon previous methodology because they accounted for the individual's sleep-wake cycle. However it is not exactly clear what aspect of HPA axis functioning is being measured during that window of observation because cortisol levels during the first hour after awakening reflect two distinct components of HPA axis activity: pre-awakening levels and the cortisol awakening response (CAR). The increase in cortisol levels that is typically observed within the first hour after awakening defines the CAR. It is a discrete, distinct and independent component of HPA axis activity that is not just a continuation of the pre-awakening rise in cortisol levels. The CAR is considered to be a true response to awakening (Wilhelm et al. 2007, Hellhammer et al. 2009), that is under distinct genetic influence (Würst et al. 2000; Bartels et al. 2003; Kupper et al. 2005), and which is also distinct from, and independent (Edwards et al., 2001) of other components of the diurnal cortisol

rhythm. Functionally, the CAR is thought to reflect adaptation to stress (Pruessner et al. 2003), a potential indicator of psychosocial adjustment in children (Boyce and Ellis, 2005), and may thus be a more appropriate index than overall post-awakening levels for testing the relationship between post-awakening HPA axis activity and psychosocial factors (Chida and Steptoe, 2009).

To date, the only study linking the CAR to conduct problems found that girls with pure externalizing behavior problems had a greater CAR than girls with comorbid internalizing behavior problems and girls without behavior problems but found no association in boys (Marsman et al. 2008). Other studies that included the CAR (Popma et al. 2007; Fairchild et al. 2008) failed to show any associations with conduct problems. Once again, methodological considerations may underlie these negative findings. Measurements of the CAR are especially sensitive to participant compliance (Kudielka et al. 2003; Broderick et al. 2004). The Fairchild et al. (2008) study relied on adolescents with conduct disorder to self-sample their salivary cortisol which is problematic because non-compliance is characteristic of individuals with conduct disorder (see Chamberlain & Patterson, 1995; Loeber et al. 1993; McMahon & Forehand, 2003) Additionally, Popma et al. (2007) and Marsman et al. (2008) only measured the CAR on one day, whereas Hellhammer et al. (2007) cautions that multiple days of measurement are required to obtain a reliable estimate of the CAR because the CAR on any one day is heavily influenced by situational factors. Therefore it is possible that conduct problems in boys could be associated with the CAR when it is measured more reliably (by parents and on more than one day).

There is also a need to further elucidate the mechanisms by which cortisol may be related to conduct problems. In this respect, the CAR is of particular interest

because it has been associated with negative affect (whereas underlying baseline waking levels are not; Polk et al. 2005; Ellenbogen et al. 2006; Steptoe et al. 2007; Stetler and Miller, 2005) which in turn has been associated with conduct problems (Loney et al. 2006; Séguin et al, 2002). This suggests the hypothesis that an attenuated CAR is a marker for emotional dysregulation in boys which when manifested as negative affect may lead to conduct problems in the same way that other aversive states do (Anderson and Bushman, 2002). Therefore in this study, we tested whether negative affect could be mediating the potential CAR – conduct problems relationship.

Finally, we also tested whether sex differences play a role in the CAR -- conduct problems relationship and the CAR -- negative affect relationship. It has been reported that the cortisol – conduct problem relationship is present in boys but not in girls (Shirtcliff et al. 2005; Smider et al. 2002) and that girls have a positive while boys have a negative relationship between cortisol levels and conduct problems (Sondeijker et al. 2007; Dorn et al. 2009). Additionally, an association between the CAR and negative affect was observed in males but not in females (Polk et al. 2005). Both the CAR and HPA axis activity in general are reported to be differently related to a wide variety of psychosocial factors in males and females such as attention problems (Susman et al. 2007), sensation-seeking (Rosenblitt, 2001), social cognition under stress (Smeets et al. 2009), decision making after a stressful task (van den Bos et al., 2009), violence exposure in urban youth (Kliewer, 2006) and callous-unemotional traits (Loney et al. 2006).

In sum, we expected that: a) the level of conduct problems will be negatively associated with the CAR; b) the level of negative affect will be negatively associated

with the CAR; c) these effects will be stronger for boys than for girls; and d) negative affect will mediate the conduct problems – CAR relationship.

Materials and methods

Participants

The participants in this study were recruited from a large longitudinal sample. Participants were solicited initially via the Live Birth Register of Québec's Ministry of Health as part of a survey of infant development (Santé Québec et al., 1997). This community cohort was drawn from two cities, Montréal (80%) and Québec City (20%). Briefly, the original sampling plan targeted 1000 families that lived in the vicinity of the two cities. There is a 16-week age range between the oldest and youngest child in the sample to reduce age-related variance in the data.

L'Institut de la Statistique du Québec assessed 572 toddlers at age 5 months. As of the age 17 months data collection, they kept priority access to 123 families and Séguin and colleagues maintained yearly follow up of the 449 remaining families. Of these remaining families, 43% participated in this data collection wave at age "10" ($\underline{M}$ = 10.62 years; SD = 0.12 years; N = 190) and we obtained cortisol samples on 33% (N = 148). Of these, 46% (n = 68) were girls and 54% (n = 80) were boys. At the birth of their first child, mothers were aged $\underline{M}$ = 27.1 years, and when the child was aged 5 months 2.7% of families were headed by a single parent, 9% of mothers had not completed high school while 34.5% held a university degree and 8.3% of families reported an income lower than Cad $30 000 whereas 29% reported incomes higher than Cad $60 000. The low income cut-off, or 'poverty line', for a typical family of two living in an urban environment was Cad $21 414 at the time these data were collected (Statistics Canada, 1997). Parental consent was obtained at each time

point of the study, in accordance with the ethics standards of the Ste-Justine Hospital Research Center and the American Psychological Association (2002).

Conduct Problems

Children's mothers completed questions assessing the frequency of various problem behaviors. Only a few of the mothers (5.3%) elected to complete English questionnaires while the remainder (94.7%) completed their questionnaires in French. Language of questionnaire was not significantly related to any of the dependent or independent variables used in this study. The behavior measures were developed for the National Longitudinal Survey of Children and Youth (Statistics Canada, 1996). The abbreviated, items and their sources are as follows: 1) Breaks/damages his/her own things; 2) Steals things; 3) Rebels/refuses to obey; 4) Fights; 5) Breaks/damages other's things; 6) Lies/Cheats; 7) Physically- attacked others; 8) Vandalizes 9) Hits/Bites/Kicks other kids [questions 1-9 from the Ontario Child Health Study (see Offord, Boyle and Racine, 1989)]; 10) Overreacts angrily to accidents; 11) Scares others to get what he/she wants; 12) Reacts aggressively when contradicted; 13) When teased strikes back; 14) Reacts aggressively to things being taken away [questions 10-14 from Dodge and Coie, 1987)]; 15) Doesn't change conduct after being punished; 16) Throws tantrums/angers quickly [questions 15 and 16 from the National Longitudinal Survey of Children and Youth; see Statistics Canada (1996)]. Items were rated never, sometimes, often. We summed these items to create a conduct problems scale score ($\alpha = .85$).

Negative Affect

Children's mothers also rated their child's negative affect using the following items from the Ontario Child Health Study (see Offord et al. 1989): a) Seemed sad or

unhappy; b) Wasn't as happy as other kids; c) Had difficulty having fun. Items were rated never, sometimes, often. Our scale ($\alpha = .67$) was created by summing the scores.

Saliva sampling and biochemical analysis

Cortisol levels were assayed from saliva samples, collected by parents, on the Monday and on the Friday following a nurse's home visit, unless they were not school days, in which case, saliva was sampled on the next closest school day. This is because children often have higher cortisol concentrations on school days than non-school days (Davis et al. 1999). Cortisol in saliva reliably reflects the unbound fractions of circulating cortisol that are capable of interacting with cortisol receptors in the brain (Laudat et al. 1988). Prior to cortisol collection, nurses visited the children's homes and instructed parents in obtaining saliva samples. Parents were requested to collect saliva samples just after children woke up, then 30 minutes after. The difference between the second and first daily cortisol measurements were averaged over both days to create the CAR scores. Parents were also instructed to record the time of sampling regardless of whether they were able to comply with the instructions on timing. This was done to control for the actual time of day at sampling, because cortisol secretion follows a diurnal rhythm with a 10-fold variation in concentration across the day (Goodyer et al. 2001). Also, parents were instructed to not to let their children consume caffeine, sweet juices, dairy products or a major meal before the morning saliva was sampled because these things are thought to reduce the reliability of saliva sampling (Kudielka et al. 2003).

Salivary cortisol concentrations were measured using a sensitive enzyme immunoassay kit (Salimetrics, State College, PA, USA) as specified in the kit

instructions. Briefly, 25 µl of standard or saliva was incubated with assay buffer and conjugate in the antibody-coated well for 1hr at room temperature. All assays were done in duplicates. After several washes, assay plates were incubated with the color-developing reagent for 30min at room temperature (protected from light). Three minutes after stopping the reaction, plates were shaken and the optical density of each well was read on a spectrophotometer set at 450nm and 492nm. The difference in optical density between the two wavelengths was used to calculate salivary cortisol concentration using the Assayzap software program (Biosoft Inc.). The limit of detection of this assay was 0.012 µg/dl for a range of 0.012-3 µg/dl. The intra- and inter-assay coefficient of variation was 2.14% and 4.00%, respectively.

Data Analysis

At each cortisol sampling time point, outliers in the distribution (n = 9) that were also true hyper-secretors (n = 7), defined as being above the median for over 75% of measurements, were reassigned a value equaling two standard deviations above or below the mean following the procedure of Kertes and Gunnar (2004). The remaining two participants with outlying scores who were not true hyper-secretors were discarded. In order to ensure the validity of our CAR score, which is highly sensitive to participant compliance (Kudielka et al. 2003, Broderick et al. 2004), twenty nine participants were excluded because the second daily measurement was taken less than 30 minutes or more than 70 minutes after awakening or if they failed to record one or more sampling times (n = 18). Two more participants were discarded for having 4 or more questions missing on the conduct problems scale. Participants who were missing fewer items had their missing scores replaced by the mean. In total, out of 148 participants, we discarded 51 participants leaving 97 (45 girls and 52

boys). None of the included participants' were using steroid medications according to their mothers' reports. The excluded participants were not significantly different from the non-excluded participants for age, family income, family type (single parent vs. intact/recomposed), mother's level of education, age of mother at the birth of her first child, child's conduct problems or child's negative affect.

Pearson's correlations were conducted separately for boys and girls to assess the strengths of the relationships between the CAR scores on the one hand and either negative affect or conduct problems on the other. After this, we tested whether boys' and girls' correlation coefficients were significantly different from each other for the above-mentioned relationships. To further verify whether our gender group differences were significantly greater than differences from randomly assigned groups, we conducted two permutation tests with 999 resamples using the S+ Resample library developed by Hesterberg et al. (2005) for the S-PLUS language (Insightful corp, 2007). Permutation tests resample participants into groups and compare the resampled statistics to the statistic of interest from the original data. They are non-parametric significance tests that provide accurate p-values even when distributions are non-normal and for these reasons, permutation tests are considered the "gold standard" (Hesterberg et al. 2005; also see LaFleur and Greevy, 2009, for more explanation of permutation testing).

Finally, to test whether negative affect was a mediator in boys' conduct problems-CAR relationship, we used Preacher and Hayes' (2008) meditational (indirect effects) bootstrapping analyses macro for SPSS (SPSS Inc., 2006). This method is similar to the classic Sobel test (see Sobel 1982/1986) for mediation, however because it is non-parametric and is thus more robust in the face of non-

normal sampling distributions, it tends to be more powerful especially when sample sizes are not large (see MacKinnon et al. 2002/2004). Following Preacher and Hayes' recommendations to verify our causal assumptions, we also ran a reverse-mediation test.

Results

Gender differences and the CAR

The CAR was not related to time of awakening. There was no significant difference in the CAR ($\underline{M}$ = .061, $\underline{SD}$ = .10 for boys µg/dl; $\underline{M}$ = .042, $\underline{SD}$ = .11 µg/dl for girls) between boys and girls. Our CAR values were similar to those reported in studies of early and mid-adolescent boys and girls (Ellenbogen et al. 2006; Marsman et al. 2008; Fairchild et al. 2008). There was also no difference in negative affect between boys and girls ($\underline{M}$ = 4.54, $\underline{SD}$. = 1.16 for boys; $\underline{M}$ = 4.49, $\underline{SD}$ = 1.36 for girls). As expected, boys tended to have higher conduct problems scores than girls ($\underline{M}$ = 22.21, $\underline{SD}$. = 4.29 for boys; $\underline{M}$ = 20.74, $\underline{SD}$ = 3.61 for girls; $t = -1.87$; $p < .05$, one-tailed). The relationships between CAR and conduct problems and CAR and negative affect are shown below in Figures 1 and 2, respectively.

The significant associations for boys, reported in Figures 1 and 2, remained significant even after controlling for maternal depression, body mass index, and demographic variables. Other potential confounds thought to affect cortisol levels, such as awakening time, Ritalin use, abnormal mood or stress at time of cortisol sampling and the use of steroid-based medication had no effect on cortisol measures.

There were significant differences in the correlation coefficients between boys and girls for the relationships between CAR and conduct problems ($p = .016$) and

CAR and negative affect (p = .033). Permutation tests, done with 999 resamples yielded positive results both for the CAR and conduct problems relationship (p = .02; one-tailed) and the CAR and negative affect relationship (p = .038; one-tailed).

Mediation Analyses

Negative affect was found to completely mediate the relationship between boys' CAR and conduct problems as seen in Figure 3 below.

Finally, a reverse-mediation test was conducted in order to provide support for our causal assumptions as suggested by Preacher and Hayes (2008). This test is similar to normal mediation testing except that the hypothesized mediating variable, negative affect, is switched with the hypothesized dependent variable: conduct problems. The results of this test were different from the previous one, in that the C path which was non-significant in the previous test is now significant (p = .012) which provides some support for our causal assumptions (Preacher and Hayes, 2008). However, our study is still correlational in nature and thus we cannot claim causality.

Discussion

The current study investigated the relationships between the CAR, conduct problems and negative affect in boys and girls. Results from this study can be summarized as follows. The relationships between the CAR and conduct problems and the CAR and negative affect differed between boys and girls. Boys but not girls with a lower CAR had greater negative affect and more conduct problems. Our results, in conjunction with the Pruessner et al. (2008) finding of an attenuated CAR in males but not females with recent onset psychosis, suggest that males may be more vulnerable to the problems associated with an attenuated CAR. Most importantly, this study helps to elucidate the mechanisms by which HPA axis functioning is related to

conduct problems with our finding that negative affect fully mediates the CAR – conduct problems relationship, suggesting that an attenuated CAR is a marker for emotional dysregulation in boys which when manifested as negative affect may lead to conduct problems.

Mediation

This study shows for the first time that negative affect fully mediates the cortisol – conduct problems relationship. This ties in to theories of arousal and conduct problems (see sensation-seeking theory, Eysenck, 1964; Zuckerman, 1979) which propose that low HPA axis activity reflects physiological under-arousal, which is experienced as an aversive state and predisposes individuals to display conduct problems. Our finding is also concordant with Susman et al. (1987)'s hypothesis that emotions may be the mechanism by which the effects of hormones are exhibited in antisocial behavior. Additionally, it has been suggested that the CAR reflects one's ability to adapt to the social environment (Brosnan et al., 2009; see also Pruessner et al. 2003); thus perhaps boys with a low CAR cannot 'fit in' socially, possibly leading to negative affect and, in turn, conduct problems. Not 'fitting in' socially could be frustrating and Berkowitz' theories (1980/1993) state that frustration and negative affect can lead to aggression as aversive states are known to do (Anderson and Bushman, 2002).

However, our results may contrast with those of Shoal et al. (2003), who reported that low morning cortisol predicted higher levels of aggression in males five years later, an effect that was mediated by low levels of self-control and not by negative emotionality. We note that Shoal et al. (2003) used the negative emotionality super factor of the self-report multidimensional personality questionnaire (Tellegen,

1982), which is composed of stress reaction, alienation and aggressive subscales. This concept of negative emotionality is very different from our negative affect scale, assessed by mother-report, which is comprised of items that mainly assess sadness. Also Shoal et al. (2003) only used one morning measurement of cortisol and did not record participants' wake-up times. Because cortisol secretion follows a diurnal rhythm with a 10-fold variation in concentration over the day (Goodyer et al. 2001) cortisol sampling must be timed in relation to an individual's sleep-wake cycle in order to obtain a reliable measure. Furthermore, studies (Polk et al. 2005; Ellenbogen et al. 2006; Steptoe et al. 2007; Stetler and Miller, 2005) point specifically to the increase after awakening being associated with positive or negative affect while the underlying baseline levels are not; thus the differences in which components of HPA axis function were studied may explain the different results.

Conduct Problems

This study shows for the first time that the CAR is associated with conduct problems in boys. Our results are an extension to the many studies on HPA axis activity and conduct problems reporting negative relationships between cortisol levels and conduct problems (McBurnett et al. 2000, 2005; Vanyukov et al. 1993; Pajer et al. 2001; van de Wiel et al. 2004; Shirtcliff et al. 2005; Smider et al. 2002; Yu and Shi, 2009; Dorn et al. 2009; Popma et al. 2007; Fairchild et al., 2008; Snoek et al., 2004; van Goozen et al. 1998, 2000; Shoal et al. 2003). This study adds a new facet to our understanding of the HPA axis – conduct problems relationship as it has found another distinct component of HPA axis activity which is a biological marker for conduct problems in boys.

Negative Affect

Our finding of an inverse relationship between the CAR and negative affect in boys but not girls may help to clarify contradictory findings linking the CAR to emotional states (for a review see: Chida and Steptoe, 2009). Polk et al. (2005) found that the CAR is positively associated with negative affect in men but not in women and Steptoe et al. (2007) found that positive affect measured by brief self-reported ratings of positive affect at multiple pre-determined time points, but not by questionnaire, was negatively associated with the CAR in a sample of men but found no relation with negative affect. On the other hand, Ellenbogen et al. (2006) reported that cortisol levels 60 minutes after awakening (a related measure to the CAR) are positively associated with positive affect in adolescents, and Stetler and Miller (2005) reported that a blunted CAR was associated with mild to moderate depression in a population sample of women not taking anti-depressants.

Methodological issues may contribute to the inconsistencies. For instance, the studies associating a low CAR with either reduced positive affect or increased negative affect generally employed more days of measurement and better CAR sampling procedures [2 days of measurement and written confirmation of sampling time in both the Ellenbogen et al. (2006) and this study; three days of measurement and compliance monitoring in the Stetler and Miller, (2005) study] than studies associating a low CAR with reduced negative affect [one day of measurement in a hotel during a potentially stressful virus challenge study in the Polk et al. 2005 study, and two days of measurement and written confirmation of sampling time in the Steptoe et al. 2007 study]. It is also possible that the inconsistent results can be reconciled by considering the possible moderating roles of age and sex, since reduced

positive or increased negative affect is associated with a low CAR in male children (this study) adolescents of both sexes (Ellenbogen et al. 2006) and adult women (Stetler & Miller, 2005) whereas reduced negative affect is associated with a low CAR only in adult men (Polk et al. 2005; Steptoe et al. 2007).

The above explanations make the questionable assumption that positive and negative affect are opposites and may not be found together. Indeed, controversy surrounds this issue with some assuming that they are opposites while others maintain that they are independent (Feldman Barrett, & Russell, 1999). While none of the above-mentioned studies examined this, Polk et al.'s (2005) pattern of results suggested that positive and negative affect had opposite directions of association with cortisol measures while Ellenbogen et al. (2006) found similar directions of association between positive and negative affect on the one hand and cortisol measures on the other (though not all of these associations were significant).

Limitations

One limitation of our study is noted in that although we asked parents to record their compliance with cortisol sampling procedure, we did not electronically monitor compliance. The CAR is especially sensitive to participant compliance and non-compliance may more often result in erroneously lower than erroneously higher CAR scores (Kudielka et al. 2003; Broderick et al. 2004). Therefore it is possible that our results can be explained by parents who are less compliant with experimental procedures having sons but not daughters with greater negative affect and more conduct problems. However, non-compliance as measured by missing samples, missing records of sampling times, not sampling within the critical period or missing responses for multiple questionnaire items was unrelated to boys' behavioral or

emotional problems. Nevertheless, future studies should employ electronic methods of compliance monitoring so as to increase confidence in the validity of the CAR measures. Additionally, since we used the same raters to assess negative affect and conduct problems, perhaps their strong association is due to shared method variance. So parents with children who display conduct problems might attribute more negative affect to those children than is warranted. We would have been able to circumvent this limitation to some extent by using data from another informant – typically the teacher. Unfortunately, teachers were on strike during the year in which cortisol data was collected and thus we were only able to use one rater.

Summary

In the present study, negative affect was found to fully mediate the CAR-conduct problems relationship in boys. These results lend support to the theory that low levels of cortisol are linked to unpleasant states in boys and that the magnitude of negative affect evoked contributes to the tendency to display conduct problems. Additionally we found gender differences in the relationships between the CAR and both negative affect and conduct problems. Specifically, boys but not girls with a low CAR had both higher levels of negative affect and conduct problems. The strength of these relationships in girls was not significantly different from zero but was significantly different from those of boys, suggesting that different psychobiological mechanisms contribute to conduct problems in boys as compared to girls.

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Figure 1

Scatter plot of the relationships between the CAR and conduct problems for boys (n = 52) and girls (n = 45)

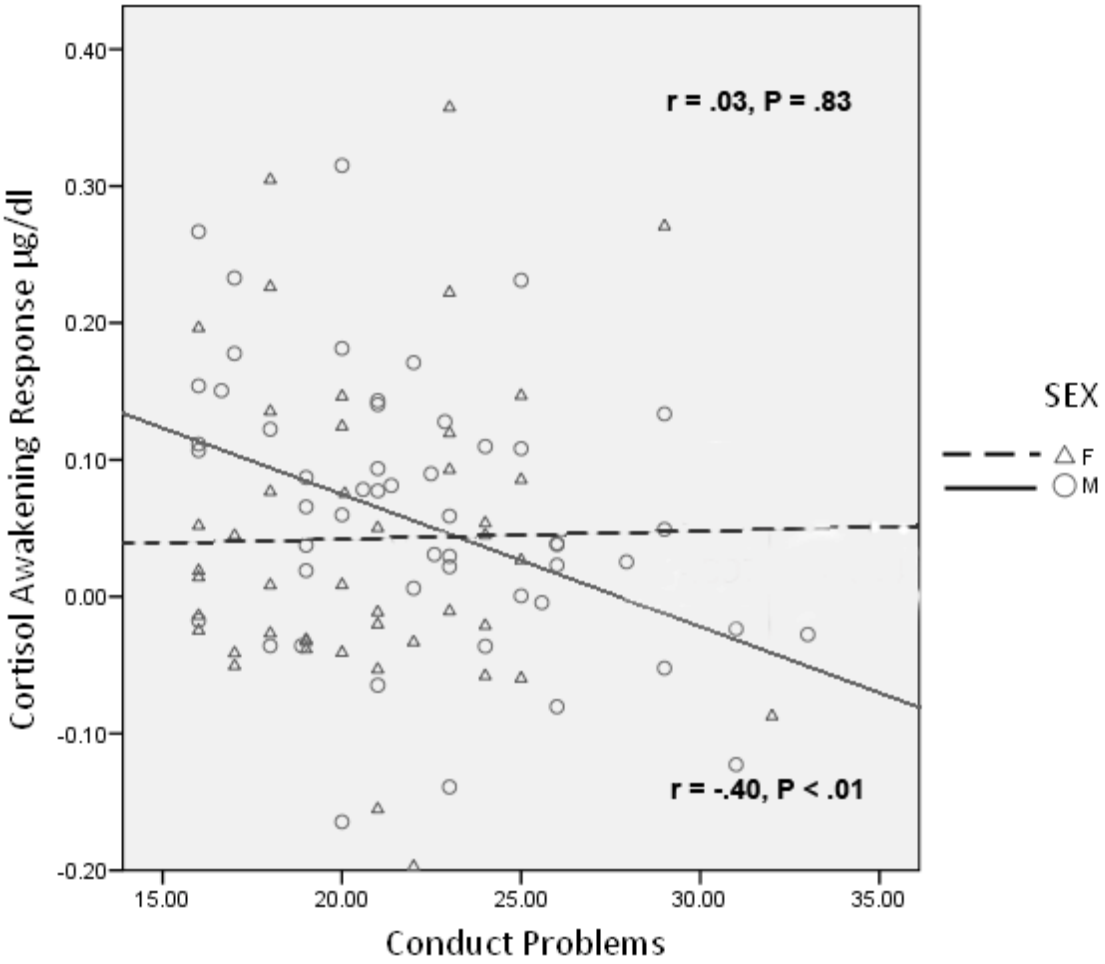


Figure 2

Scatter plot of the relationships between the CAR and negative affect for boys (n = 52) and girls (n = 45)

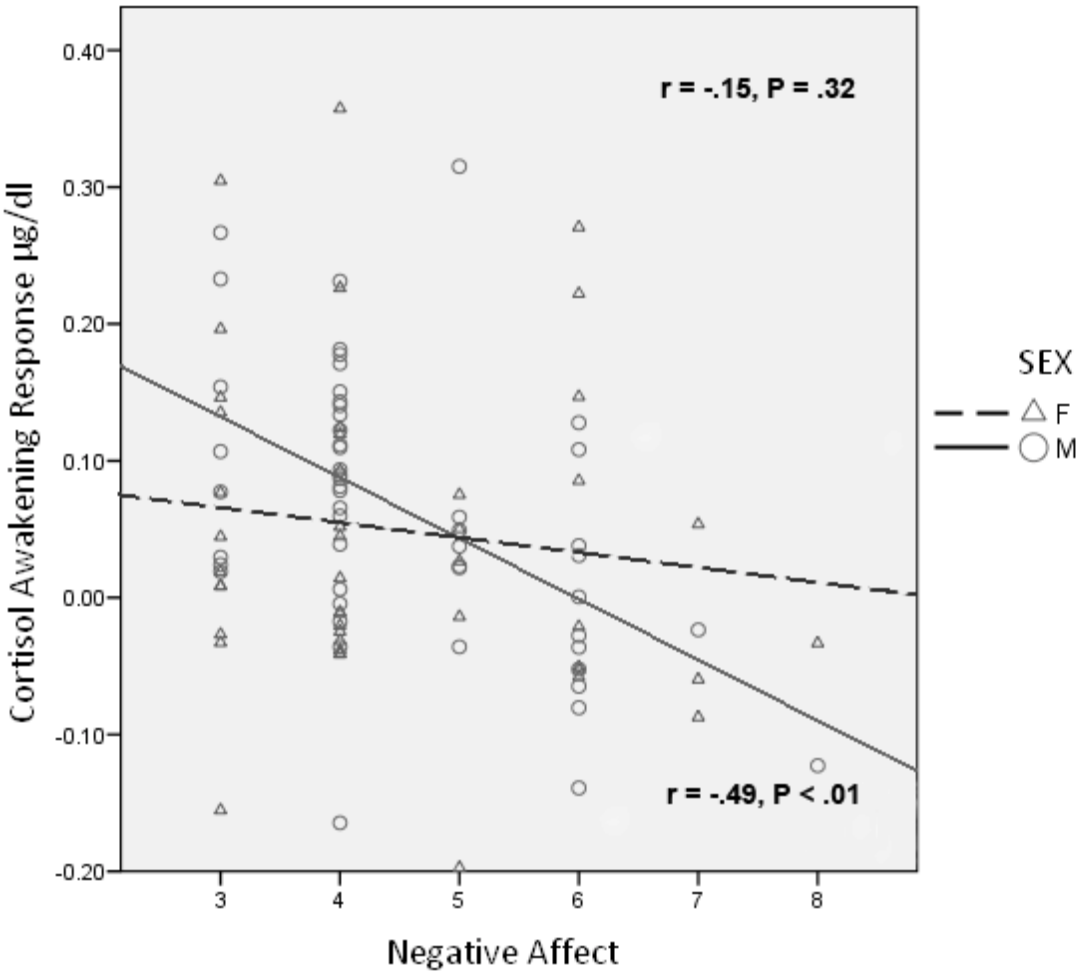
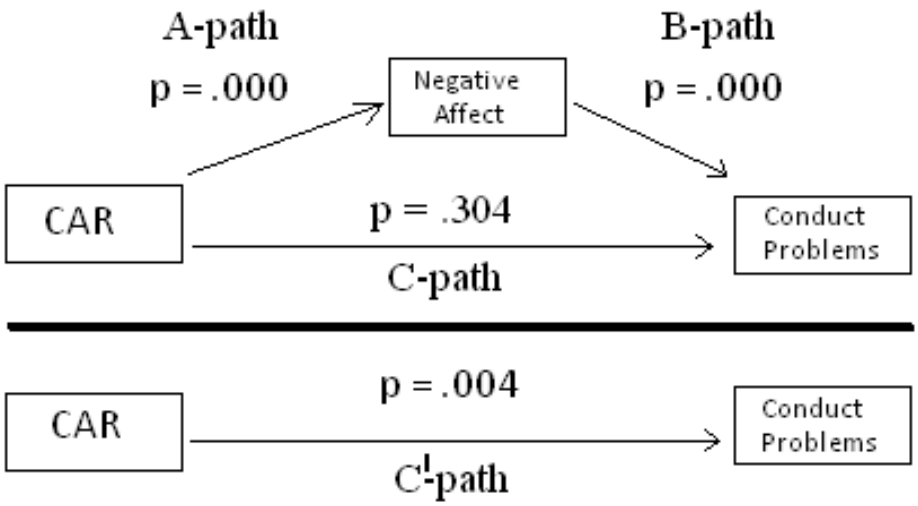


Figure 3

Diagram of A, B, C and C^{l} paths from indirect effects analysis



Déclaration de l'étudiant concernant les articles

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Anthony Walsh, Sonia J. Lupien, Richard E. Tremblay and Jean R. Séguin

Negative affect mediates the relationship between the cortisol awakening response and conduct problems in boys

Article en phase finale de préparation. La revue ciblée est *Hormones and Behavior*

Déclaration

In the research and writing of the article I played the major role in the selection of the topic and measures to be analyzed, the selection of analysis strategies and methods, the analysis of the data, the interpretation of the results and the writing of the article. I was not involved in the planning of the collection phase, the collection of data itself and the data entry.

Sonia J. Lupien consulted with me in the pre-analysis phase, the analysis phase and reviewed some drafts. She helped with the selection of measures, strategies and methods of analysis and the interpretation of results. Her lab assayed our saliva samples and I made presentations to her lab in the pre-analysis and analysis phase and received feedback. She planned the cortisol collection phase and was a co-investigator on the grant that served to fund the cortisol sampling.

Richard E. Tremblay was the nominated principal investigator on the grant that served to fund the cortisol sampling. He has reviewed the article draft included in the thesis.

Jean R. Séguin, my supervisor, played an important role in all the major phases of this research including the planning of the collection phase, the data analysis phase and the writing phase. He also directed funding towards this research.

Signature:_____

Date:_____

Chapter 6: Discussion

Overview

The main finding of this research is that negative affect mediates the negative relationship between the CAR and conduct problems in boys. This final chapter discusses the implications of this and other findings of the featured study for theory and future research. First, the featured study and its findings are summarized. Second, the implications are discussed. Third, the sex difference findings are further explored and a new theoretical model is presented to explain them. Finally the thesis is concluded with brief summary and discussion of important research questions generated by the featured study.

Summary

The goal of this research was to further understanding of the psychobiological processes related to conduct problems. Specifically, it sought to clarify the relationship between stress-regulating mechanisms and conduct problems in youth. The research question was: *what mechanism accounts for the relationship between stress regulation and conduct problems?* The study investigated one stress-regulating mechanism, the cortisol-awakening response (CAR), and one potential mediating mechanism, negative affect, in a population sample of ten-year-olds. The hypotheses were that: a) the level of conduct problems will be negatively associated with the CAR; b) the level of negative affect will be negatively associated with the CAR; c) these effects will be stronger for boys than for girls; and d) negative affect will mediate the conduct problems – CAR relationship. All of these hypotheses were

supported. This study helped to elucidate the mechanisms relating stress regulation and conduct problems and has thus furthered our understanding of the psychobiological processes related to conduct problems. It also found a new biological marker for conduct problems in boys. There are many implications for psychobiological theories of conduct problems, yet many unanswered questions remain, both old ones and new ones generated by this research. These will be addressed by this discussion and hopefully also by future research.

Implications for psychobiological theories of conduct problems

The two main psychobiological theories of conduct problems which relate to stress-regulating mechanisms are fearlessness theory (Raine, 1993) and sensation-seeking theory (Eysenck, 1964; Zuckerman, 1979). Of these two theories, our results tie in slightly with sensation-seeking, which supposes that low levels of arousal are felt as an aversive state. While fear may be considered a type of negative affect, our measure included only the following questions a) Seemed sad or unhappy; b) Wasn't as happy as other kids and c) Had difficulty having fun. Although we did not test for fear, our measure does test for aversive states. However, we did not include measures of sensation-seeking and so we can only support the notion that low cortisol is associated with an aversive state, though only in boys. However, our results suggest that psychobiological mechanisms other than those proposed by sensation-seeking and fearlessness theories may play a role in the expression of conduct problems. For instance, aversive states such as negative affect may contribute to conduct problems by increasing irritability and frustration (see frustration-aggression theory, Berkowitz, 1980; Berkowitz, 1993). Indeed, Berkowitz hypothesizes that the degree of negative

affect experienced is proportional to the probability of behaving aggressively (Berkowitz, 1993). Aversive states are thought to lead to conduct problems without requiring the mediating mechanism of an increased sensation-seeking drive (Anderson & Busman, 2002). Thus by combining elements of sensation-seeking theory with frustration-aggression theory, a new theory is possible whereby low levels of cortisol increase negative affect which in turn increases frustration, thereby leading to anti-social behaviour. This is concordant with Susman et al. (1987)'s hypothesis that emotions may be the mechanism by which the effects of hormones are exhibited in anti-social behaviour. Additionally, it has been suggested that the CAR reflects one's ability to adapt to the environment (Brosnan et al., 2009; see also Pruessner et al. 2003) and thus perhaps boys with a low CAR cannot 'fit in', possibly leading to negative affect and, in turn, conduct problems.

Future researchers should examine whether negative affect exclusively mediates the CAR – conduct problems relationship or whether it also mediates other HPA axis – conduct problems relationships as well. While the findings that measures of affect seem to be associated with the CAR but not underlying waking levels (Polk et al. 2005; Ellenbogen et al. 2006; Steptoe et al. 2007; Stetler and Miller, 2005; and this study – data not reported) support the former possibility, future investigation is still warranted, partly because elevated evening cortisol is often associated with depressive symptoms (for a review see Burke et al., 2005). Additionally, in order to better understand which mechanisms link which components of HPA-axis functioning to conduct problems, future studies should attempt to simultaneously test different potential mediators such as sensation-seeking (see sensation-seeking theory, Eysenck, 1964; Zuckerman, 1979), fearlessness (see fearlessness theory, Raine,

1993), frustration (see frustration-aggression theory, Berkowitz, 1980), low self-control (see Shoal et al. 2003), ability to adapt to the environment (Brosnan et al., 2009) and negative affect. Thus there is a need for future studies on conduct problems to include multiple measures of HPA axis functioning as well as multiple potential mediators.

Finally, it is possible that another factor may be involved, namely acute and chronic stress exposure during early childhood. Some authors (de Kloet, 2003; Sapolsky et al., 2000) hypothesize that exposure to chronic or occasional but intense stress can result in down-regulation of the HPA axis (for a review of cortisol hyposecretion findings in neglected and abused children see Golier & Yehuda, 1998). In one study, trauma-exposed individuals, who developed post-traumatic stress disorder (PTSD), were found to have attenuated CARs as compared with those who did not develop PTSD and healthy controls (Wessa, Rohleder, Kirschbaum, & Flor, 2006). Additionally, another study observed attenuated CARs in adults with chronic fatigue syndrome who reported serious childhood trauma (Heim et al., 2009). Thus it is possible that an attenuated CAR is a marker of childhood trauma which is both associated with negative affect (see Egeland, 1991) and conduct problems (Widom, 1989; Lang, Klinteberg, & Alm, 2002). Interestingly, the two other studies which measured the CAR and assessed childhood trauma found the opposite relationship to the studies mentioned above (Weissbecker, Floyd, Dedert, Salmon, & Sephton, 2006; Gonzalez, Jenkins, Steiner, & Fleming, 2009); however they used exclusively female participants, suggesting that childhood trauma may have differential effects on stress-regulating mechanisms in males and females. Perhaps males will more often

become desensitized to stress whereas females become more sensitive. This may explain why males tend to develop more externalizing problems and females tend to develop more internalizing problems (see Angold, & Rutter, 1992; Lewinsohn, Hops, Roberts, Seeley, & Andrews, 1993; Zahn-Waxler, 1993).

Gender Differences

The present research found sex differences in both the CAR – conduct problems relationship and the CAR – negative affect relationship: males had negative associations between these measures and females had no associations between these measures. Additionally, the present research found that boys displayed more conduct problems than girls. Indeed, gender differences in the expression of conduct problems are widely reported (for instance, see Archer, 2004 for a review of sex differences in aggression). Explanations for these findings include differences in socialization processes in that biological influences on behaviour may be magnified in boys who are socialized to 'express' and attenuated in girls who are socialized to 'suppress' (for reviews see Block, 1983; Rury, 1987; Bussey, & Bandura, 1999; Udry, 2000; Zelezny, Chua, & Aldrich, 2000), as well as the influence of sex hormones such as estrogen (see Sondeijker et al., 2007; also see chapter III for further explanation). Additionally, the primary male sex hormone, testosterone, may interact with cortisol to influence the expression of conduct problems. For instance, Popma, Vermeiren, et al. (2007) found that afternoon levels of cortisol moderate the effect of testosterone on aggression in young adolescent boys referred to a delinquency diversion program. It was found that for boys with low but not high levels of cortisol, testosterone was positively related to aggression. It is therefore possible that a combination of both low

levels of cortisol and high levels of testosterone is required for conduct problems to be manifested. A brief discussion of testosterone as it relates to conduct problems is thus warranted.

Testosterone.

Testosterone facilitates aggression when administered to adults (Kouri, Lukas, Pope, & Olivia, 1995; Pope, Kouri, & Hudson, 2000). In a meta-analysis, examining 45 studies (N=9760), Book, Starzyk and Quinsey (2001) concluded that testosterone had a small positive association with aggression levels. Pre-natal exposure to testosterone masculinizes the brain, differentiating male from female neural architectures (Book et al.). In humans, the extent of such exposure is associated with increased assertive and aggressive behaviour (Turner, 1994) as well as increased rough and tumble play and dominant behaviour (Archer, 1991). According to the organizational-activational hypothesis (Phoenix, Goy, Gerall, &Young, 1959) as it relates to gonadal hormones such as testosterone, pre and perinatal exposure has an organizational effect on the central nervous system affecting development and functioning, and may also sensitize or desensitize the individual to hormonal circulation later in life (Leshner, 1978). During and after puberty, exposure has a mainly activational effect producing contemporaneous effects on behaviour (Rubin, Reinisch, & Hasket, 1981; Young, Goy and Phoenix, 1964). In their meta-analysis, Brook and colleagues found the strongest associations between testosterone and aggression levels for the 13-20 year old range. This is consistent with the organizational-activational hypothesis but may also be easily explained in other ways. For instance, because higher testosterone concentrations are associated with earlier

puberty and thus physical maturity and increased musculature in boys, the stronger relationship between testosterone and aggression may be at least be partly due to the moderating effect of such physical changes, which would increase the likelihood of winning an aggressive confrontation and thus the motivation to aggress. Additionally, since the highest group levels of testosterone and aggression were observed during this age range, the weaker relationships observed for other age ranges may be at least partially due to range restriction effects which reduce effect sizes (Brook et al.).

A longitudinal study of males measuring testosterone at ages 13, 16 and 21 found that hormonal concentrations were positively related to aggression, delinquency and social dominance but that these relationships differed at different ages (van Bokhoven et al., 2006). At age 13, testosterone was most strongly related to leadership, at age 16 it was most strongly related to aggression and at age 21 it was most strongly related to delinquency. This may be explained by referring to Mazur and Booth's (1998) suggestion that testosterone represents dominance seeking which may take different forms depending on the social conext. According to Mazur and Booth "Often dominance is expressed nonaggressively. Sometimes dominant behavior takes the form of antisocial behavior, including rebellion against authority and law breaking". Since the social context varies with age, this may explain the differential associations of testosterone with the behaviours reported above (van Bokhoven et al.).

Original Hormonal Theory of Conduct Problems.

Whereas it is thought that the mechanism linking high levels of testosterone to conduct problems is dominance-seeking (Mazur and Booth, 1998), there is less

consensus on the mechanism linking low levels of cortisol to conduct problems. In the featured study, we found that negative affect fully mediated the cortisol – conduct problems relationship suggesting that an attenuated CAR reflects an abundance of negative affect, which can lead to conduct problems. In light of this, we propose the model (see Table 1 below) in which conduct problems in males result from a combination of negative affect, an attenuated CAR being the hormonal correlate, and dominance-seeking, high testosterone being the hormonal correlate. When dominance-seeking is high but negative affect is not, then children seek dominance in prosocial ways such as leadership. Indeed, Rodkin et al. (2000) have identified both antisocial and prosocial subtypes of socially dominant boys in the 4th to 6th grade classroom setting.

Table 1

Model of dominance-seeking and negative affect interaction

		Dominance Seeking	
		Low	**High**
Negative Affect	**High**	Negative Non-Dominant Behaviours ex: 'self-harming'	**'Negative' Dominant Behaviours ex: Bullying**
	Low	'Non-negative Non-Dominant Behaviours ex: Affiliating with groups	'Non-negative' Dominant Behaviours ex: Leadership

Sex differences in the relationship between the CAR and negative affect may be related to the organizational effects of testosterone. Indeed, the organizational influences of gonadal hormones during the pre and perinatal periods may orient an individual towards certain types of emotional responding (Marcus, Maccoby, Jacklin, & Doering, 1985). Such organizational effects are thought to account for sex differences in the frequency, intensity and age patterns of emotional behaviours such as boys demonstrating more fearlessness, anger and frustration. It is possible that these organizational effects interact with activational effects of cortisol to increase boys' tendency to display negative affect when cortisol levels are low. In sum, it is proposed that males, who tend to be exposed to higher levels of pre and perinatal testosterone, are more susceptible to the contemporaneous effects of a low CAR on negative affect. This effect may be responsible for all the gender differences observed in the featured study. Dominance-seeking is influenced by organizational and activational effects of testosterone so that early exposure orients the individual towards dominance seeking and later exposure produces contemporaneous increases in dominance seeking. Negative affect then interacts with dominance-seeking so that individuals with high dominance-seeking and low negative affect seek dominance pro-socially, such as through leadership, whereas individuals with high dominance-seeking and high negative affect seek dominance anti-socially, resulting in high levels of conduct problems.

Future research should examine this proposition by simultaneously measuring the CAR, testosterone levels, dominance-seeking and negative affect in males and females. Cortisol and testosterone can be assayed from the same saliva samples while the psychosocial factors can be measured by questionnaire. Although

not firmly established, assessing the organizational effects of testosterone may also be achieved by measuring the 2D:4D finger digit ratio which can be obtained by placing the hand of the individual in a photocopy machine and making a copy (this can be done at any age; for a review see McIntyre, 2006).

Conclusion

This thesis began with a research question: *what mechanism accounts for the relationship between stress regulation and conduct problems?* The present study found that negative affect mediates the relationship between the CAR and conduct problems in boys. In all likelihood, negative affect as a mechanism does not entirely account the relationship between stress regulation and conduct problems; nevertheless the research question has been at least partially answered. As is commonly the case, the present research has perhaps generated more questions than answers. The questions include: *why is the CAR-negative affect association found in boys but not girls? What mechanism links the CAR to negative affect? And, does negative affect mediate other cortisol – conduct problems relationships?* The first question is addressed with a model proposed in this chapter; however all three questions necessitate future studies to properly address and hopefully answer.

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