Neuroendocrinological factors of antisocial behaviour in adolescents

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Over the past two decades, research on the role of biological factors in antisocial behaviour has made vast progress. In this article, recent findings from a series of studies performed in the Netherlands on HPA-axis functioning and antisocial behaviour in male adolescents are presented. The three studies discussed here focus on diurnal variation of cortisol, cortisol reactivity to stress, and patterns of interaction between cortisol and testosterone in relation to aggression. The results of these studies are used to discuss possible repercussions on future clinical practice of biological studies on antisocial behaviour. (*Netherlands Journal of Psychology*, 63, 126-135.)

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Antisocial behaviour by children causes major suffering at the individual level since children displaying such behaviour are at risk of a series of negative outcomes in adulthood, for example criminal behaviour, social isolation, unemployment, and psychiatric disorders including depression, anxiety disorders and substance abuse (Maughan & Rutter, 2001). Moreover, it constitutes a major public health problem, with these children costing society at least ten times as much as children who develop well (Scott, Knapp, Henderson & Maughan, 2001) and with antisocial behaviour bearing the potential to cause serious trauma in victims. Currently, although short-term effectiveness of intervention strategies aimed at addressing psychosocial risk factors of antisocial behaviour (e.g. parent management training, cognitive behavioural therapy) has been demonstrated (Kazdin, 2000), the long-term effectiveness of current treatment options is limited (Offord & Bennett, 1994).

Although antisocial behaviour can be partly explained by psychosocial factors (Rutter, Giller & Hagell, 1998), research over the past decades has increasingly shown the importance of taking into account neurobiological mechanisms as well (Raine, 1993). Therefore, contemporary theoretical models of antisocial behaviour comprise both social and biological factors, reflecting the assumption that both types interplay in a complex fashion to influence the development and persistence of antisocial behaviour (Cacioppo, Berntson, Sheridan & McClintock, 2000).

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The clearest examples of findings supporting such a theoretical framework have been those showing that having a biological or a social risk factor alone predicted low rates of antisocial behaviour whereas having both factors predicted exponentially increased rates of antisocial behaviour (Caspi et al., 2002). Conversely, having a social risk factor for developing antisocial behaviour may be compensated by a biological protective factor and vice versa. As such, current research, and influential theories deriving from it, shy away from biological determinism, but do stress the need to study biology as one of the important correlates of antisocial behaviour (for a review see Raine, 2002). In order to improve the currently existing models, it is warranted to study specific potentially important factors and their relation to antisocial behaviour in detail.

Researchers have started to study relationships between various specific biological factors and antisocial behaviour, predominantly in the research fields of brain imaging (Yang & Raine, 2007), genetics (Jones & Viding, 2007), and psychophysiology/endocrinology, which is the focus of this article. A series of studies that have been conducted over the past few years in Amsterdam, the Netherlands will be outlined and discussed. In these studies, the relationship between (re)activity of the hypothalamus-pituitary-adrenal (HPA) axis, the most important neuroendocrinological stress-regulating system in humans, and antisocial behaviour was investigated in non-clinically referred delinquent adolescent boys. First, some general theoretical background will be provided and the current relevant literature will be addressed. Second, three of the above-mentioned series of studies and their results will be outlined and discussed. Finally, potential repercussions for future clinical practice and directions for future research will be discussed. In doing so, the aim is to exemplify a specific approach to studying relationships between specific biological factors and antisocial behaviour for those professionals interested in this subject. In addition, an effort is made to provide a starting point reference for those professionals interested in starting new biologically inspired studies on antisocial behaviour.

Theoretical background

The two main biological stress-regulating mechanisms in humans are the neuroendocrinological HPA axis and the physiological autonomous nervous system (ANS). Both systems manifest a certain level of activity in resting situations, and react to either physical or psychological stress with increased activity in order to help a person to deal with upcoming challenges. Activity of the HPA axis is reflected in levels of its final product cortisol, the main stress hormone in humans. In reaction to stress, corticotrophin-releasing hormone (CRH) is released from the paraventricular nucleus in the hypothalamus. This initiates a neuroendocrinological cascade, with CRH stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary. In turn, ACTH binds to receptors in the outer cortex of the adrenal glands, resulting in the secretion of the steroid hormone cortisol (figure 1). Interestingly, this hormone can be measured in saliva in a stress-free way. The ANS works alongside and interacts with the HPA axis in regulating stress. Therefore, it has been studied simultaneously with the HPA axis activity in some previous studies. Its main peripheral parameters are heart rate and skin conductance level.

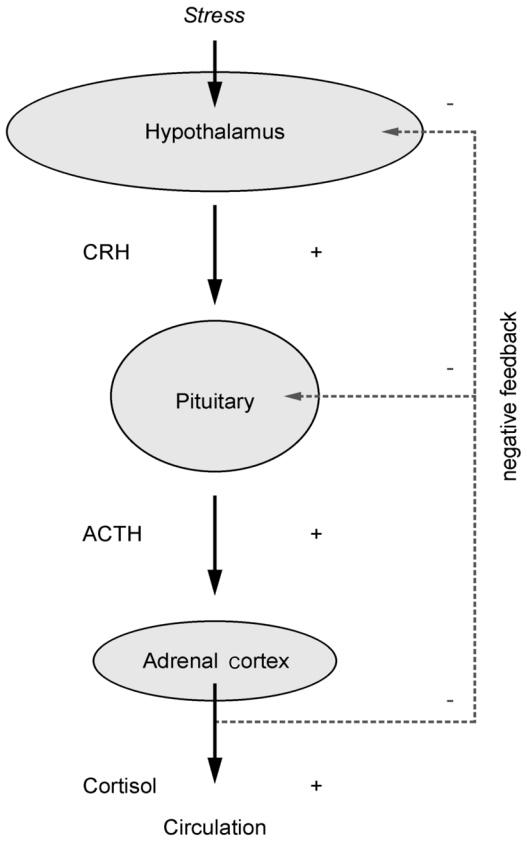

Figure 1
HPA axis.

With respect to research on antisocial behaviour, HPA axis and ANS activity has predominantly been studied within the paradigm of the low arousal theory. Within this theory, patterns of low arousal have been postulated to be characteristic of antisocial behaviour, which may be explained in two ways. A first interpretation is imbedded in the fearlessness theory, which claims that low arousal levels are markers of low levels of fear (Lykken, 1957; Raine, 1993). According to this theory, children with low arousal levels are more likely to engage in delinquent acts, physical fights and other forms of antisocial behaviour because they do not fear the negative conse-

quences of their aggressive actions. In other words, without a normal reaction of the biological stress regulating systems there will be a lack of fear conditioning. This interpretation therefore also implies that social cues, such as punishment, applied when raising or treating a child, will not be effective in children with low arousal levels. A second interpretation is provided by the sensation-seeking theory (Zuckerman, 1979), in which it is argued that low arousal levels represent an aversive physiological state. As such, individuals with low arousal levels are motivated to seek out stimulation in order to raise their arousal levels to an optimal or normal level. In this view, engaging in antisocial behaviour constitutes a way of seeking stimuli. Importantly, both interpretations are not necessarily mutually exclusive, as a person with low levels of arousal may seek out stimulation, which may result in a blunted HPA and ANS response and altered fear sensation. Moreover, a pattern of seeking out stimulation may lead to blunting of responses to stress by habituation and thereby lead to reduced fear sensation.

Literature

When preparing for the series of studies presented in this article, the available literature was first reviewed. In line with the low arousal theory, low levels of ANS parameters, i.e. heart rate and skin conductance level, have fairly consistently been associated with antisocial behaviour (see Ortiz & Raine, 2004 for a review). However, with respect to HPA activity, even though findings from previous studies have started to converge towards an association between low levels of its final product cortisol and antisocial behaviour, inconsistencies and lacunae continued to exist (Azar, Zoccolillo, Paquette, Quiros, Baltzer & Tremblay, 2000; McBurnett, Lahey, Rathouz & Loeber, 2000; McBurnett, et al., 2005). Some of these inconsistencies may be partly explained by previous studies predominantly relying on a single cortisol sample only. As such, these studies did not adequately take into account diurnal variation in cortisol levels due to the profound circadian rhythm of HPA axis activity, with high peaking levels after waking up and diminishing levels over the rest of the day. In addition to this issue concerning basal cortisol levels, the existing literature had several lacunae with respect to the association between HPA response to stress and antisocial behaviour. At present, studies in antisocial samples investigating cortisol levels in response to stress are scarce. The few previous studies on this topic have predominantly been performed in clinically referred populations with a disruptive behaviour disorder (DBD; Van Goozen, Matthys, Cohen-Kettenis, Buitelaar & Van Engeland, 2000). As such, it had been left to discussion whether findings of low HPA activity in these samples may be extrapolated to non-clinical populations of subjects with antisocial behaviour problems. Moreover, it remained unknown whether low HPA reactivity is specific for pathological levels of antisocial behaviour problems (i.e. having a DBD diagnosis), or whether it is also associated with less serious levels of antisocial behaviour. Furthermore, definitions of antisocial behaviour have varied largely between studies, with definitions being predominantly broad, without distinguishing subtypes of antisocial behaviour. As such, studies investigating specific subtypes of antisocial behaviour in relation to these biological parameters were warranted, to investigate which specific behavioural aspects of antisocial behaviour are most clearly related to arousal parameters. Finally, when studying biology-behaviour relationships, important information may be obtained by investigating several parameters simultaneously. For example, only few studies to date have measured both HPA and ANS in relation to antisocial behaviour. Apart from providing information on whether disturbances in one parameter accompany the other, simultaneous measurement of parameters allows for studying interaction effects. This may be interesting since it has been postulated that some inconsistencies in the current literature on single hormone-behaviour relationships may have been caused by such effects between various hormones. For example, cortisol and testosterone have been hypothesised to interact in relation to aggression (Viau, 2002).

The current series of studies

To help address some of these issues, a series of studies on HPA (re)activity were conducted in a sample of non-clinically referred delinquent male adolescents applying rigorous methodology. In this article, results from three specific studies will discussed. In study 1, multiple samples of cortisol were collected during the day. In study 2, cortisol, heart rate, and skin conductance were simultaneously measured during a standardised stressor. Finally, in study 3, interaction effects between cortisol and testosterone in relation to specific forms of aggression were investigated. The study population consisted of 12 to 14-year-old male adolescents referred to a delinquency diversion programme in Amsterdam (DP group; $n = 114$). In the Netherlands, children between 12 and 18 years of age who have committed a minor offence can be sent to a diversion programme to prohibit court intervention. This option is only available for specific types of petty offences: wanton destructiveness, vandalism, simple theft, hooliganism, infractions on the firework regulations and minor forms of aggression. All participants, and one of their parents, were interviewed with the Diagnostic Interview Schedule for Children (DISC), version IV (Shaffer, Fisher, Lucas, Dulcan &

Schwab-Stone, 2000). Participants meeting criteria for either an oppositional defiant disorder (ODD) or a conduct disorder (CD), or both, were classified as having a disruptive behaviour disorder (DBD). Subjects from the study population were divided in a subgroup of delinquents with a DBD diagnosis (DP+; $n = 35$) and a subgroup of delinquents without a DBD diagnosis (DP-; $n = 79$). DP boys were compared with a matched normal control group (NC = 32) of boys without police contacts who were recruited from local schools and football clubs. Delinquent boys and normal controls were matched group-wise for age, IQ , socioeconomic status and ethnicity.

In study 1, the cortisol diurnal curve, including cortisol levels during the first hour after awakening (cortisol awakening response; CAR), was investigated and compared between a subgroup of DP+ subjects, a subgroup of DP- subjects, and a NC group. As such, this study aimed to extend previous research on the cortisol-antisocial behaviour association in adolescents by sampling cortisol repeatedly over the day. In the first 30 minutes after awakening, mean cortisol levels increase 50 to 100%, remaining elevated for at least one hour. Therefore, the CAR has been postulated to be superimposed on the underlying diurnal pattern, comprising aspects of basal activity as well as dynamic changes during the first hour after awakening (Clow, Thorn, Evans & Hucklebridge, 2004). It was found that the DP+ group, but not the DP- group, had significantly lower overall cortisol levels in the first hour after awakening as compared with the NC group. Furthermore, the DP+ group showed a significantly slower decrease in cortisol during the diurnal cycle than the NC group. These findings suggest a more rigid HPA axis in the DP+ group, resulting in a flattening of the diurnal cortisol cycle. Moreover, a gradual decrease in cortisol levels during the CAR was observed from the NC over the DP- to the DP+ group (i.e. from normal behaviour to serious antisocial behaviour problems). In line with this finding, a significant, though modest, inverse correlation between delinquency scores on the Child Behaviour Checklist (CBCL) and overall cortisol levels in the first hour after awakening was found. Notably, no significant associations were observed between parameters of antisocial behaviour and afternoon or evening cortisol levels (Popma, Doreleijers, Jansen, Van Goozen, Van Engeland & Vermeiren, 2007a).

Using the same method to divide subjects into subgroups, study 2 compared groups with respect to HPA and ANS reactivity to a standardised, and video recorded, public speaking task (PST; Jansen, Gispen-de Wied & Kahn, 2000). This stressor was chosen because it induces anxiety and has proven to be an effective stressor in both children and adults (Dickerson & Kemeny, 2004; Kudielka, Buske-Kirschbaum, Hellhammer & Kirschbaum, 2004). It embodies all the major criteria of an anxiety-arousing situation, for it is perceived as a threat, only partially under participant control and implies uncertainty with regard to the outcome and/or consequences. Parameters consisted of cortisol, heart rate, skin conductance level, and self-reported negative affect, which were all measured before, during and after the stressful task. Again, the DP+ group differed significantly from the NC group. In the present study, the DP+ group showed a blunted cortisol and heart rate reaction to the PST as compared with normal controls, in spite of similar total scores of self-reported negative affect during the stress task. As such, the current results may suggest an alteration in the coordination of emotions and psycho-physiological mechanisms during stress in delinquent boys with a DBD diagnosis. The main implication of this study, though, is that low HPA/ANS reactivity to a fearful social stressor may be a marker for distinguishing delinquent boys with a DBD diagnosis from both normal controls and delinquent boys without a DBD diagnosis. In addition, the current results extend previous findings in clinical samples to a non-clinical sample (Popma et al., 2006a).

In study 3, an effort was made to explore the interplay between several hormones, by investigating both cortisol and testosterone in relation to two subtypes of aggression. More specifically, the interaction between cortisol and testosterone in relation to overt and covert aggression was studied. Overt aggression (feeling angry and displaying aggression by e.g. arguing and fighting) is hostile, openly defiant and likely to be impulsive and poorly controlled, while covert aggression (feeling angry without expressing openly) is furtive, hidden and more controlled (Buss & Durkee, 1957; Lange, Dehghani & De Beurs, 1995). An interaction effect between cortisol and testosterone was found in relation to overt aggression, such that a significant relationship between testosterone and overt aggression was only present at low levels of cortisol but not at high levels of cortisol. These results indicate a moderating effect of cortisol on the relationship between testosterone and overt aggression in delinquent male adolescents (Popma et al., 2007b).

Discussion

Summarising, the presented studies showed the following results. It was found that the delinquent boys with a DBD diagnosis (DP+ group), but not the delinquent boys without a DBD diagnosis (DP- group), had significantly lower overall cortisol levels in the first hour after awakening, but not during the rest of the day as compared with the NC group. Furthermore, the DP+ group showed a significantly slower decrease in

cortisol during the diurnal cycle than the NC group and showed a blunted cortisol response to a standardised stressor. Finally, an interaction effect between cortisol and testosterone was found in relation to overt aggression, such that a significant relationship between testosterone and overt aggression was only present at low levels of cortisol but not at high levels of cortisol.

It remains speculative why the inverse association between cortisol and antisocial behaviour was only found for morning cortisol levels and not for afternoon or evening levels. One explanation may be associated with cortisol levels being highest in the morning, with levels varying largely between individuals (Pruessner et al., 1997). As such, due to large inter-individual variance in morning cortisol levels, possible correlations between cortisol and behavioural measures may become apparent more easily when sampling at this time of day then when measuring during the trough of the cortisol cycle (i.e. in the afternoon and evening). Another plausible explanation may be that levels of cortisol during the first hour after awakening do not merely reflect basal HPA functioning, but also HPA reactivity (Edwards et al., 2001). Therefore, low cortisol levels in the first hour after awakening may reflect a blunted HPA response to waking up. In line with the idea that antisocial behaviour is associated with altered HPA reactivity, and not so much with shifts in basal cortisol levels, the DP+ group showed a slower decline in cortisol levels during the day, in comparison with the NC group. These findings suggest that, possibly because of a smaller amplitude of the morning cortisol peak, feedback mechanisms controlling HPA axis activity are also less effective, resulting in smaller and more sluggish variations in cortisol levels during the day.

A second way in which the presented studies extended previous research was by measuring HPA/ANS response to a standardised stressor in relation to antisocial behaviour, in a non-clinically referred sample of delinquent adolescents. Whereas the above-mentioned findings of altered HPA (re)activity in antisocial behaviour from study 1 were based on cortisol samples collected during the day, while participants went about their normal daily routine, in study 2 HPA and ANS reactivity to a laboratory psychosocial stressor was investigated.

From a theoretical perspective, the findings from study 2 may well fit into the fearlessness theory, assuming low arousal levels to reflect low levels of fear. As such, children with low arousal levels are more likely to engage in delinquent acts, physical fights and other forms of antisocial behaviour because they do not fear the negative consequences of their antisocial actions. In line with this assumption, the DP+ groups showed a blunted cortisol and heart rate response to a fearful stressor as compared with normal controls. In contrast, the current findings do not seem to support the sensation-seeking theory. This theory argues that low arousal levels represent an aversive physiological state that makes individuals seek out stimulation in order to raise their arousal levels to an optimal or normal level. First, no differences between groups were found for resting levels of HPA/ANS parameters. Second, since the HPA/ANS response to an anxiety-provoking stressor was blunted (and even hardly noticeable) in the DP+ group, there was no evidence for such stress raising arousal levels to 'a more optimal level'.

A third way in which the present findings extended the literature was by studying the interaction between cortisol and testosterone in relation to overt and covert aggression. In the third presented study, a positive association between testosterone levels and overt aggression was found when cortisol levels were low, but not when cortisol levels were high. First, this interaction may be explained by cortisol being related to psychological variables (e.g. social withdrawal, behaviour inhibition) that in turn influence the testosterone - aggressive behaviour relationship (Dabbs, Jurkovic & Frady, 1991). Second, high cortisol levels may be directly protective against aggression, as suggested by the first and second study presented in this article, reporting an inverse relationship between antisocial behaviour and cortisol. A third explanation may be that cortisol directly influences the effects of testosterone (e.g. at the receptor level) and thereby influences the association between testosterone and overt aggression (Viau, 2002).

Finally, since previous findings in favour of an association between low HPA reactivity and antisocial behaviour were mainly derived from studies in clinical samples of DBD youth, the current studies extended these findings to a non-clinically referred delinquent sample. Since both low cortisol levels during the cortisol awakening response (CAR) and during a fearful stressor were specifically found in delinquent boys with a DBD diagnosis, these aspects of HPA functioning may be of value in distinguishing delinquent boys with serious behaviour problems who require psychiatric treatment, from boys with less serious behavioural problems who do not require treatment. Moreover, the finding of an inverse association between dimensional scores of antisocial behaviour and cortisol levels during the CAR suggests the inverse association between HPA reactivity and antisocial behaviour to extend from non-pathological levels of antisocial behaviour to pathological levels.

Limitations

The findings of the presented studies need to be valued in the light of some limitations. First, in all the studies, saliva was sampled on one day only. Measuring cortisol on two or more consecutive days has been advised, to allow controlling for day-to-day variations (Wust, Wolf, Hellhammer, Federenko, Schommer & Kirschbaum, 2000). Second, in study 1, participants sampled saliva at home without electronic monitoring. Although a number of precautions were taken to avoid non-compliance, and no evidence was present for differences in compliance between groups, some influence of non-compliance may still have been present (Broderick, Arnold, Kudielka & Kirschbaum, 2004). Third, in study 2, levels of HPA and ANS parameters during the stress protocols were not compared with a non-stress protocol in order to control for non-specific variations in levels. As such, it remains possible that the observed increases in biological and psychological parameters were not solely due to the applied stressor. Still, with respect to the public speaking task, this particular stressor has been shown to be highly effective (Dickerson & Kemeny, 2004; Kudielka, Buske-Kirschbaum, Hellhammer & Kirschbaum, 2004), and a significant increase in cortisol, heart rate and negative affect was found during the stressor, with a subsequent decrease after finishing the PST. Moreover, in order to specifically include the response to the applied stressor in the statistical analyses, increases in cortisol and heart rate levels were corrected for baseline values measured before the start of the stressor. Fourth, in study 3, only a single testosterone sample was obtained, not allowing for a mean of several samples to be taken, which could have minimised the effect of pulsatility, as was done for cortisol. Also, testosterone was stored at -20 °C, which has recently been shown to be inferior to storing at -80 °C (Granger, Shirtcliff, Booth, Kivlighan & Schwartz, 2004). Fifth, comorbidity and experience of adverse life events was not taken into account. In the present studies, the National Institute of Mental Health (NIMH) Diagnostic Interview Schedule for Children (DISC), version IV (Shaffer et al., 2000) was used to assess psychopathology. This is an extensive structured psychiatric interview of which both the child and parent versions were administered by trained interviewers. For reasons of relevancy, and due to time constrictions, it was chosen to focus on the sections on attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD) and post-traumatic stress disorder (PTSD). However, since previous studies have shown comorbidity of internalising disorders to be prevalent in delinquent samples (Vermeiren, 2003) and possibly of influence on HPA functioning (Van Goozen et al., 2000), future studies on antisocial behaviour should take into account comorbidity of such disorders. Furthermore, although PTSD was assessed, a more detailed questionnaire on adverse life events would have rendered more valuable information on stressful experiences. Although no subjects were diagnosed with PTSD it is likely that the delinquent sample experienced more adverse life events as compared with the normal controls (see also below).

Clinical implications

Before discussing the clinical implications of the present findings, it needs to be noted that, despite the recent progress in our understanding of the relationships between biological factors and antisocial behaviour, major lacunae still exist. Moreover, the translation of knowledge from studies comparing groups to the individual patient in clinical practice must be undertaken with care. For example, in the current studies, large between-subject variations in basal cortisol values and cortisol response to stress were found within separate subgroups. As such, although low morning cortisol and a blunted cortisol response to fearful stressors may be specific for groups of delinquent boys with DBD (DP+), this biological profile is not necessarily characteristic for all individual subjects within this subgroup. Moreover, this pattern may also be found in some individuals without a DBD diagnosis, whether delinquent or not. Bearing in mind these nuances, it is still possible and important to extrapolate the present findings to various aspects of future clinical practice (Popma & Raine, 2006b). Clinical implications of the current findings may be discussed with respect to four different aspects of clinical practice: diagnostic identification, providing treatment options, predicting relapse, and treatment evaluation (Grisso & Zimring, 2004).

With respect to diagnostic identification, studies presented in this article observed low cortisol levels in the first hour after waking up and a blunted HPA/ANS response to various stressors in delinquent boys with a disruptive behaviour disorder (the DP+ group). As such, these characteristics may be an additional tool to diagnose those individuals within heterogeneous delinquent groups with the most serious antisocial behaviour problems. In addition, specificity of diagnostic assessment may be enhanced by performing both a psychiatric assessment and a cortisol profile, for example to distinguish DBD boys with altered HPA activity patterns from those with a more average pattern.

Taking into account cortisol profiles in diagnostic processes may also help to increase specificity and effectiveness of current treatment options for youngsters with a disruptive behaviour disorder. Preliminary evidence for this assumption was reported by Van de Wiel et al. (2004), who

studied cortisol reaction to a stressor in 22 clinically referred DBD disordered children before psychotherapeutic treatment. They found that low cortisol response during stress predicted poor treatment outcome. As such, the subgroup of children with this biological profile might need other forms of treatment than those with a normal cortisol response to stress. Replication of this finding in clinical samples with a larger N and in non-clinical samples is warranted.

Apart from directing specific treatment options to specific groups of patients, new treatment possibilities may arise from biological and biosocial studies. Although controversial, influencing a biological factor that is related to antisocial behaviour may in turn modulate antisocial behaviour. For example, as discussed above, low arousal has been related to antisocial behaviour. There is evidence that stimulants, e.g. methylphenidate, both increase arousal and reduce aggressive behaviour (Connor, 2002). Importantly, however, taking into account biological vulnerabilities in understanding juvenile antisocial behaviour can also lead to new approaches for non-pharmacological interventions. With respect to HPA activity, preliminary evidence for the potential of non-pharmacological programmes to alter biological vulnerability for antisocial behaviour has been provided by Fisher and Stoolmiller (2002) in a study evaluating a foster care intervention programme. A group of aggressive juveniles were found to have a flattened diurnal pattern of cortisol levels before entering the programme. After the intervention, diurnal cortisol patterns were found to be more normal, with high cortisol levels in the morning and a decrease during the day, while aggression levels had diminished.

In a similar fashion as for treatment options, relapse prediction may also be influenced by knowledge of HPA activity patterns. For example, in a longitudinal study on recurrence of depression after treatment, cortisol levels were measured after remittance of a depressive episode. Heightened cortisol levels were found to predict a new episode of depression. In a similar fashion, biological parameters may be useful in predicting reoccurrence of antisocial behaviour. Still, studies investigating this hypothesis are currently lacking.

The last aspect of clinical practice that may be informed by assessing HPA/ANS activity patterns is treatment evaluation. If a certain biological profile that is correlated with behavioural problems can be assessed before treatment, investigating this same biological profile again after treatment may be useful as a measure of treatment outcome. As discussed above, preliminary results have suggested that successfully diminishing aggressive behaviour by means of a foster care intervention programme coincided with normalisation of diurnal cortisol patterns (Fisher & Stoolmiller, 2002). As such, cortisol levels may be a parameter that could inform practitioners on treatment efficacy.

Recommendations and directions for future research

The present findings provide evidence for a subtle and complex association between low HPA reactivity and antisocial behaviour and bring forward some recommendations for future research. First, future studies need to take into account time of day when sampling cortisol and preferably collect samples during the first hour after awakening as well as during standardised and theoretically relevant stressors. Second, measuring of other biological parameters simultaneously (e.g. other arousal regulating mechanisms or other hormones thought to play a role in antisocial behaviour) was proven to be of value. Third, with respect to interactions of cortisol with other hormones in relation to aggression, future studies should not only investigate testosterone but also other androgens, for example DHEA and DHEAS. Several studies have found these androgens to be related to aggression, sometimes even more strongly than testosterone (Van Goozen, Matthys, Cohen-Kettenis, Thijssen & Van Engeland, 1998).

Although the studies presented help to extend the literature in several ways, numerous questions have been left unanswered. In the current studies, the sample consisted of boys only. This recruitment strategy was chosen primarily because boys of this age are more likely to conduct delinquent behaviour and therefore are more likely to be caught by the police and sent to the delinquency diversion project. As such it was expected not to be feasible to recruit enough girls to study them as a separate group. However, this remains an important issue for future studies, since previous findings of HPA functioning in antisocial adolescent girls have been conflicting (Pajer, Gardner, Rubin, Perel & Neal, 2001; Azar, et al., 2004). Moreover, it seems likely that gender differences in the relationship between cortisol levels and antisocial behaviour are present since rates and types of antisocial behaviour differ between boys and girls (Vermeiren, 2003) and cortisol levels have been found to differ and show a gender-specific developmental pattern during puberty and adolescence (Spear, 2000). Also, research has shown that males and females differ in their neuroendocrine responses to stress and in sensitivity to different types of stressors (Stroud, Salovey & Epel, 2002). Taking into account that females are more prone to develop internalising disorders, such as depression, anxiety and eating disorders, and males tend to develop antisocial behaviour problems, gender may also be a moderating factor in ex-

plaining variation in psychopathology outcome resulting from early adverse experiences (Van Goozen, Fairchild, Snoek & Harold, 2007).

At present, much remains unknown as to why low HPA (re)activity may be related to antisocial behaviour. Several theories have been postulated. First, genetic influences may underlie this association. There is increasing evidence that antisocial behaviour may be partly explained by genetic influences (Rutter, Silberg, O'Connor & Simonoff, 1999). Moreover, cortisol levels have been shown to be partly heritable (Bartels, Van den Berg, Sluyter, Boomsma & De Geus, 2003). However, at present the mechanisms through which genes influence cortisol levels remain largely unknown. Moreover, a large part of variety in cortisol levels can not be explained by genetic factors.

A second explanation for altered HPA functioning in antisocial individuals may be provided by the fact that these individuals frequently experience stressful events. Low HPA activity has been hypothesised to reflect an adjustment to extreme or chronic stress (Heim & Nemeroff, 2001; Gunnar & Vazquez, 2001). As mentioned above, one way of experiencing stressful events may result from individuals seeking out stimulation and stressful environments to raise low basal levels of arousal (a process called self-selection), which may in the long run cause habituation and low reactivity to stress. However, it is likely that children with antisocial behaviour problems also experience stress in other more uncontrollable ways, since these children often come from problematic backgrounds involving neglect and abuse (Rutter et al., 1998). In line with this assumption, patterns of HPA underactivity have been found in individuals experiencing chronic stress (Heim et al., 2001). Moreover, chronically abused and neglected children growing up in orphanages also showed flattening of diurnal cortisol patterns (Carlson & Earls, 1997). Studies of various adult psychiatric patients with a history of early childhood abuse have now revealed long-term changes in HPA axis activity (Heim & Nemeroff, 2001; Heim, Plotsky & Nemeroff, 2004).

Conducting longitudinal studies could help in studying the effects of adverse life events and could contribute to answering the question whether low HPA activity precedes antisocial behaviour or is a consequence of such behaviour. So far, most studies have examined correlations, including the ones discussed in this article, while only a limited number of studies have demonstrated that HPA and ANS activity may predict the onset and course of disruptive behaviour problems. With respect to heart rate and skin conductance, low resting levels in adolescents have been found to predict adult criminal behaviour (Raine, Venables & Mednick, 1997). With respect to HPA activity, Shoal et al. (2003) found that low resting cortisol in 10- to 12-year-old boys was predictive for correlates of disruptive behaviour problems at the age 15 to 17. However, these findings warrant replication in other samples. To help address this issue, a follow-up study will be conducted in the current sample, investigating the predictive value of cortisol levels on development and persistence of antisocial behaviour.

Conclusion

The current series of studies has aimed to contribute to extending previous findings on the link between biological parameters and antisocial behaviour. Patterns of low morning cortisol levels after awakening and low HPA/ANS reactivity to an anxiety-inducing stressor were found in delinquent boys with a DBD diagnosis as compared with controls. Moreover, a positive association between testosterone and overt aggression was found when cortisol levels were low, but not when cortisol levels were high. Taken together, these findings corroborate on previous research suggesting biological factors to be related to antisocial behaviour in children and adolescents. Obviously, such biological factors should be viewed as being in constant interplay with environmental factors when shaping behaviour in general and antisocial behaviour in particular. Moreover, it is important to understand that biology does not equal destiny. If a certain biological pattern is found in an individual today, it may have changed by tomorrow, both through environmental influences and as a consequence of biological development over time, for example during puberty. Biological factors constitute a complex but important set of keys toward increasing our understanding of the mechanisms underlying the development and persistence of antisocial behaviour. Ultimately, understanding these mechanisms should result in earlier and more effective interventions.

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