Summary

Antisocial behavior peaks in adolescence, such that the vast majority of adolescents reports to have committed some form of antisocial behavior (e.g. Junger-Tas et al., 1994). The prevalence of antisocial behavior increases with the onset of adolescence, followed by a decrease when entering adulthood (e.g. Sampson and Laub, 1992; Moffit, 1993). An extensive body of research has aimed to explain adolescent antisocial behavior, and has identified many psychosocial risk factors, such as parenting practices or peer influences, to partly explain antisocial behavior (e.g. Loeber et al., 2009). In the last decades, neurobiological factors have been increasingly studied, in order to enhance our understanding of the underlying mechanisms of antisocial behavior. One such a neurobiological risk factor is low activity of the hypothalamic-pituitary-adrenal (HPA) axis (Raine, 1993; Zuckerman and Neeb, 1979), which has frequently been related to antisocial behavior (e.g. (McBurnett et al., 2000; Popma et al., 2007b; Virkkunen, 1985). However, particularly in adolescent samples, studies yielded inconsistent results (see review by Alink et al., 2008).

These inconsistencies may in part be due to developmental changes taking place during adolescence. Firstly, adolescence is characterized by puberty-related physical and endocrinological changes (e.g. Root, 1973). It has been suggested that activity of the HPA axis, as a main endocrinological system, changes over adolescence as well (see review by Gunnar and Vazquez, 2006). Secondly, social risk factors for antisocial behavior also alter during adolescence, for instance susceptibility to influences of peers (Gardner and Steinberg, 2005). Such changes are likely to interfere in the relation between HPA axis activity and antisocial behavior. Accordingly, it is increasingly emphasized that in order to understand the development of antisocial behavior, interrelations between risk factors should be taken into account (Bassarath, 2001; Dodge and Pettit, 2003; Raine, 2002; Susman, 2006). Therefore, the aim of the current thesis was to investigate the development of antisocial behavior in relation to HPA axis activity in adolescence, thereby including interrelations with other risk factors, namely, influence of friends, other stress-related neurobiological parameters, and testosterone.

The central neurobiological risk factor for antisocial behavior in this thesis, is decreased HPA axis activity. The HPA axis is one of the main endocrinological systems in our body, involved in adaptation to changing environmental demands (e.g. McEwen, 2004). HPA axis activity is predominantly studied by examining its end-product, cortisol, the main stress hormone in humans. HPA axis activity has a basal diurnal rhythm, and responds to stressful situations by an increase in cortisol. The basal diurnal rhythm is characterized by highest cortisol levels early in the morning, which steadily decline throughout the day. Superimposed on this diurnal rhythm, is the cortisol awakening response (CAR), a distinct increase in cortisol levels occurring in the half hour following awakening, followed by a decrease. The level of the CAR is thought to reflect basal activity of the HPA axis, while the response in cortisol levels to awakening is considered to reflect the flexibility of the HPA axis (Fries et al., 2009).
The two low arousal theories offer a neurobiological explanation for the relation between low HPA axis activity and antisocial behavior. First, the sensation (or stimulation) seeking theory states that low arousal constitutes an aversive physiological state. In order to increase (normalize) one’s arousal levels, youths may seek sensation through antisocial behavior (Zuckerman and Neeb, 1979). Second, the fearlessness theory states that low arousal reflects fearlessness, as a result of which youngsters do not fear the negative consequences of antisocial behavior (Raine, 1993).

Associations between low HPA axis activity and antisocial behavior have been confirmed rather consistently by studies in children (Alink et al., 2008), and also by the few studies in adult samples (King et al., 1990; Virkkunen, 1985; Woodman et al., 1978). Studies in adolescent samples in contrast, have shown inconsistent results (see review by Alink et al., 2008). Three possible explanations why studies in adolescent samples specifically have shown inconsistent results, are; 1) the possibility that HPA axis activity develops during adolescence, 2) the heterogeneity in persistence and type of adolescent antisocial behavior, and 3) interrelations with other factors. Investigating these topics will aid to a better understanding of the relation between HPA axis activity and antisocial behavior in adolescence.

First, adolescence is characterized by major physiological changes, which are largely endocrinologically orchestrated (Root, 1973). Based on cross-sectional studies, it is increasingly believed that HPA axis activity as well, changes with the onset of puberty and throughout adolescent development. Specifically, it is thought that the level of HPA axis activity increases with maturation (for review see Gunnar and Vazquez, 2006). This has however not previously been investigated longitudinally, hence, it is unclear whether HPA axis activity indeed develops during adolescence, and how this relates to physical maturation. Gaining such information is warranted, as changes in HPA axis activity may result in age and maturation specific relations with antisocial behavior. Fundamental knowledge on the stability of HPA axis activity, and investigating whether changes occur with age or normal (physical) development, is thus essential in the interpretation of associations with antisocial behavior.

Second, antisocial behavior in adolescence is characterized by heterogeneity in persistence and type. As for persistence, with the onset of adolescence, the prevalence of antisocial behavior increases substantially, which subsequently decreases when entering adulthood (e.g. Sampson and Laub, 1992; Moffitt, 1993). Hence, the majority of adolescents shows antisocial behavior only temporarily during this period (Moffitt, 1993). A few, however, display antisocial behavior persistently. It has been suggested that this subgroup in particular is characterized by the presence of neurobiological risk factors (Moffitt, 1993; Raine et al., 2005). Previous studies, however, were almost exclusively cross-sectional in design, and could only assess the persistence of antisocial behavior retrospectively. This is less reliable than assessing behavior prospectively in a longitudinal design, which may explain the inconsistent results. Only two studies have longitudinally assessed antisocial behavior in relation to HPA axis activity, and do suggest that particularly persistent antisocial behavior is associated with low cortisol levels (McBurnett et al., 2000; Sondeijker et al., 2008). Besides heterogeneity in persistence, antisocial behavior in adolescence is also heterogeneous in type. Most adolescent antisocial behavior consists of rule-breaking, such as theft, truancy, and vandalism. Not only is rule-
breaking the most common type of antisocial behavior in adolescence, it also appears to emerge with adolescence (e.g. Stanger et al., 1997). Aggression on the other hand is most frequent in childhood, nevertheless, those who are most aggressive as children tend to be aggressive persistently into adulthood (Lahey et al., 2003). More importantly, the relative influence of risk factors for aggression and rule-breaking may differ as well, as there are indications that low HPA axis activity is more strongly related to aggression than to rule-breaking (Burt, 2012; McBurnett et al., 2000).

Third, interrelations with other factors are likely to influence the relation between HPA axis activity and antisocial behavior. This pertains to both social factors, as well as other neurobiological risk factors. As a social factor, peer influences are highly important in adolescence, and were taken into account in this study. Adolescents are, more than any other age group, susceptible to peer influences (e.g. Gardner and Steinberg, 2005). Consequently, affiliating with deviant peers is seen as one of the major risk factors for the development of adolescent antisocial behavior (Brown, 2004; Hartup and Stevens, 1997). Moreover, antisocial friendships may mediate associations between HPA axis activity and antisocial behavior. It has for instance been shown that sensation seeking, which is associated with low HPA axis activity, is also associated with affiliation with deviant friends (Yanovitzky, 2005). These deviant friends in turn, may influence the adolescent towards behaving antisocially (Thornberry et al., 1994). Also, low HPA axis activity may be specific for severe and persistent antisocial youths (Raine et al., 2005). As they already show antisocial behavior prior to adolescence, they are likely to seek friendships with adolescents who are similar in antisocial behavior (Kandel, 1978). Therefore, to better understand the development of antisocial behavior in adolescence, this important social risk factor, i.e. antisocial behavior of friends, should be investigated simultaneously with HPA axis activity.

As stress-related neurobiological risk factors, a low CAR, low stress reactivity of the HPA axis and low stress reactivity of the sympathetic-adrenal-medullary system (SAM) have individually been associated with antisocial behavior (e.g. Alink et al., 2008; Susman et al., 2010). Because these different stress-related parameters reflect distinct, yet interrelated components of the stress system, an interrelationship in explaining antisocial behavior is to be expected. With regard to the low arousal theories, a low CAR is more closely related to sensation seeking, in order to increase low trait arousal, whereas stress reactivity of the HPA axis is more closely related to fearlessness in reaction to a stressor (Van Goozen et al., 2007). However, it has been posed that frequent sensation seeking (associated with a low CAR) may lead to habituation, and eventually to a blunted stress response (and thereby fearlessness)(van Goozen et al., 2007). Furthermore, the stress response is regulated by joint activity of the HPA axis and the SAM, and it has been hypothesized that antisocial adolescents are characterized by a disturbance in the interplay between these systems (Bauer et al., 2002). To provide a more complete explanation of antisocial behavior, these different components of the stress system should be combined.
In addition to stress-related factors, another biological risk factor for aggression in particular, is a high level of testosterone. Whereas in animal studies this relation is well-established, in humans testosterone is only weakly associated with aggression. Interestingly, interactions between testosterone and cortisol have been found in relation to aggression in delinquent male adolescents. High testosterone was associated with aggression in individuals with concurrent low cortisol levels, and not in those with high cortisol levels (Dabbs et al., 1991; Popma et al., 2007a). This has been explained by the finding that high testosterone is related to approach in rewarding situations, while low cortisol is related to fearlessness (Schulkin, 2003). This specific interplay is however less clear in general population and female samples (Glenn et al., 2011; Scerbo and Kolko, 1994; Denson et al., 2012. Although the interplay between testosterone and cortisol in relation to aggression may aid in understanding its underlying mechanisms, it needs to be replicated in the general population and females before further inferences can be drawn.

To address these issues, data from a large general population study was used (RADAR; Research on Adolescent Development And Relationships). Participants in this study were 4975 adolescents of both genders, who participated in annual assessments from age 13 on. The current thesis covers three annual assessments, at participants’ ages 15, 16 and 17 years respectively. Because antisocial development was one of the main outcome variables in the study, boys and girls scoring high on teacher-reported antisocial behavior (borderline clinical score on externalizing scale of the Teacher Report Form, TRF Achenbach, 1991a) at age 11 were over-sampled (50%). Antisocial behavior was assessed each year, by self-report questionnaires on externalizing behavior, which differentiates aggressive and rule-breaking behavior (Youth Self Report, Achenbach, 1991b). Their best friends also filled out these self-report questionnaires on their aggressive and rule-breaking behavior every year. As a measure of HPA axis activity, each year, participants sampled saliva at awakening, 30 and 60 minutes later, to assess the Cortisol Awakening Response (CAR). At age 17, participants performed the Leiden Public Speaking Task (Westenberg et al., 2009). Saliva was collected before and after the speech, in which cortisol and alpha-amylase, as measures of HPA axis and SAM reactivity respectively, was assessed. Also, testosterone was assessed in the saliva sample before the speech (resting conditions).

In chapter two, we aimed to investigate the development and stability of HPA axis activity, specifically the CAR, across adolescence. Stability from ages 15 to 17 years was investigated as rank-order and mean-level stability. Rank-order stability reflects the stability of an individual’s position within the group, and was moderate for cortisol 30 and 60 minutes after awakening, but low for cortisol at awakening. This indicates that the CAR is a relatively stable measure of HPA axis activity over the course of several years. Mean-level stability reflects the stability or change in mean-levels for the whole group. Mean-levels of cortisol at awakening did not change, while the response to awakening (cortisol 30 and 60 minutes after awakening) increased over the years. After controlling for physical development the increase was no longer significant, suggesting a matura-

5 The number of participants differed per year and per study design, detailed descriptions are given per chapter.
In chapter three, the development of the CAR from ages 15 to 17 years was associated with persistence and type of antisocial behavior. Therefore, the participants were classified as persistent high aggressive ($n = 64$) or low aggressive ($n = 326$), and as persistent high rule-breaking ($n = 79$) or low rule-breaking ($n = 311$), with latent class growth analyses. The high and low classes were compared on cortisol levels at awakening, 30 and 60 minutes later, as well as on the development of these cortisol levels from ages 15 to 17. Persistently high aggressive adolescents showed decreased cortisol levels at awakening, consistently over the years, as compared to low aggressive adolescents. Cortisol levels at 30 and 60 minutes, and the development of cortisol levels over the years, did not differ between persistent high and low aggressive adolescents. Hence, despite development of the HPA axis activity over adolescence, awakening cortisol is decreased consistently over the years in persistent high aggressive adolescents. Results confirmed that low HPA axis activity is particularly related to aggression and not to rule-breaking, as no differences between adolescents showing persistent high rule-breaking and low rule-breaking were found.

In chapter four the combined influence of HPA axis activity and friends on the development of antisocial behavior is described. Because bidirectionality in influences within friendships occurs, it was examined 1) whether lower levels of HPA axis activity predict higher levels of antisocial behavior of the best friend, which in turn predicts higher adolescent antisocial behavior, and/or 2) whether lower levels of HPA axis activity predict more adolescent antisocial behavior, which in turn predicts higher levels of antisocial behavior of the best friend? This biosocial model was analyzed separately for aggressive and rule-breaking behavior, as it was expected that aggression is more strongly associated to HPA axis activity, whereas rule-breaking behavior is more strongly associated with deviant friendships. The results confirmed those in chapter three, that aggression was predicted by low cortisol levels at awakening, over and above the prediction by aggression in the previous year. In addition, when prior rule-breaking, and rule-breaking of the best friend were taken into account, low awakening cortisol predicted rule-breaking behavior as well. Moreover, adolescent rule-breaking subsequently predicted higher rule-breaking of best friends. Effects for rule-breaking were only present for adolescents who changed friends. This suggests that adolescents with low HPA axis activity are likely to change their friendships towards more rule-breaking peers. These results also indicate that interrelations between biological and social risk factors are different for the development of aggression versus rule-breaking.

In chapter five, the interplay between the CAR, as well as reactivity of HPA and SAM to stress, was investigated in relation to antisocial behavior. The combined activity as well as interactions between these stress-related parameters in relation to antisocial behavior was examined. Because stress reactivity of the HPA axis and the SAM was assessed only at age 17, this study had a cross-sectional design. Results showed an interaction between the CAR and reactivity of the HPA axis. Low HPA reactivity was associated with antisocial behavior in those with a high CAR, whereas high HPA reactivity was associated with antisocial behavior in those with a low CAR. Also, a trend towards an interaction between HPA and SAM reactivity was found; low HPA reactivity was associated