Neurobiological stress parameters in relation to disruptive behavior

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Chapter 7

Summary and general discussion

SUMMARY

As juveniles who display disruptive behavior are at risk for a series of negative outcomes later in life (Kimonis & Frick, 2010; Loeber et al., 2009b; Maughan & Rutter, 2001), an extensive body of research has focused on psychosocial factors that are related to the development and persistence of such behavior. Over the last decades, interest in neurobiological parameters has increased substantially, aiming to find additional correlates and increase understanding of disruptive behavior. In this respect, juvenile disruptive behavior has been related to decreased activity of stress-related neurobiological systems, such as the autonomic nervous system (ANS, represented by heart rate and heart rate variability (HRV)) and the hypothalamic-pituitary-adrenal-axis (HPA-axis, represented by cortisol) (Beauchaine, 2001; Raine, 2002a; van Goozen et al., 2007). A number of longitudinal studies have indicated a predictive value of neurobiological parameters for future and persistent disruptive behavior (El-Sheikh et al., 2011; Raine et al., 1995; Shoal et al., 2003). Furthermore, there are indications for complex interplays between neurobiological parameters and psychosocial risk factors in relation to disruptive behavior (Raine, 2002b; van Goozen et al., 2007). Current research and influential theories derived from these studies stress the need for continuing neurobiological research in order to reveal correlates of juvenile disruptive behavior (Popma & Raine, 2006; van Goozen & Fairchild, 2008). In order to improve the currently existing models, specific potentially important parameters and their relation to disruptive behavior will be discussed in detail in this thesis.

A starting point when studying associations between HPA-axis and ANS functioning in relation to disruptive behavior is the assumption that juveniles who display disruptive behavior show altered sensitivity to stress (van Goozen & Fairchild, 2008). When a person is confronted with a physical or psychological stressor, the body responds with activation of the main human stress regulation systems; the ANS and the HPA- axis. Heart rate, as a general measure of ANS functioning, is an indicator of activity of both the sympathetic and the parasympathetic nervous system (respectively, SNS and PNS). Several parameters reflect SNS activity, for example alpha-amylase, which can easily be obtained from saliva (Rohleder et al., 2004; Van Stegeren et al., 2006). The specific role of the PNS can be illuminated by measuring heart rate variability (HRV), also studied as respiratory sinus arrhythmia (Berntson et al., 1993; Grossman & Taylor, 2007).

Associations between these stress-regulation systems and disruptive behavior can be viewed from the perspective of various theories. First, in the *low arousal* theory, decreased ANS / HPA-axis (re)activity are regarded as a marker of fearlessness and

sensation seeking, which in turn may predispose to disruptive behavior (Raine, 1993; Raine, 2002b; van Goozen et al., 2007; Zuckerman, 1979). Second, in the *polyvagal theory*, decreased parasympathetic activity or increased parasympathetic reactivity are regarded as a reflection of emotional dysregulation, which is a hallmark for psychopathology, including disruptive behavior (Beauchaine, 2001; Porges, 1995; Porges, 2007).

An important development in this field of research is the increasing use of longitudinal data. Such designs allow for studying the predictive value of neurobiological parameters for future and persistent disruptive behavior. Studies showed that low resting heart rate, HRV and cortisol were predictive for future antisocial, delinquent or aggressive behavior throughout different age ranges (El-Sheikh et al., 2011; Raine et al., 1990; Raine et al., 1997; Shoal et al., 2003). Some studies also showed a predictive value when baseline disruptive behavior was taken into account (Raine et al., 1995; Sondeijker et al., 2008), although other studies did not (Baker et al., 2009; van Bokhoven et al., 2005a).

Furthermore, longitudinal designs can enhance insight in the stability of ANS / HPA-axis parameters and disruptive behavior, as well as the relationships between them. However, stability has mainly been studied in general population and clinical samples, it thus remains unclear to what extend ANS / HPA-axis parameters and disruptive behavior are stable in delinquent samples. Moreover, to date the relationship between the development of neurobiological parameters and the development of disruptive behavior remains understudied. One study showed that low initial HRV activity in childhood, but not development of HRV over time, was related to increasing externalizing symptoms (El-Sheikh & Hinnant, 2011). These findings need replication with additional neurobiological and behavioral parameters, as well as in other samples.

Whereas part of the previous studies examined single systems, investigating the pattern of activity in different systems involved in the stress-response is likely to shed further light on associations between stress-regulation and disruptive behavior. It has been suggested that disruptive children are characterized by a disturbance of the interplay between the different physiological systems involved in the regulation of stress (Bauer et al., 2002). This can be applied to the interplay between the sympathetic and the parasympathetic nervous system (respectively SNS, PNS), as well as to the interplay between the SNS and the HPA-axis. Indeed, some studies found concurrent low levels of SNS and PNS to be related to juvenile disruptive behavior (Beauchaine et al., 2007; Boyce et al., 2001; El-Sheikh et al., 2009). Another study found that

concurrent low activity in SNS and HPA-axis activity was associated with higher levels of aggression (Gordis et al., 2006). These results stress the importance of assessing parameters concurrently.

Most previous studies investigated heterogeneous measures of disruptive behavior, whereas a small number of studies differentiated between specific subtypes of aggression, such as proactive and reactive aggression. It is proposed that these are two qualitative different forms of aggression, that likely show distinctive neurobiological profiles (Hubbard et al., 2002). Previous studies have indeed shown differences in neurobiological correlates of proactive and reactive aggression. Proactive (or instrumental, 'cold-blooded') aggression has been related to increased resting HRV, and attenuated heart rate and HRV responsivity, whereas reactive (or emotional, 'hot-blooded') aggression has been associated with decreased HRV and increased cortisol (Lopez-Duran et al., 2009; Murray-Close & Rellini, 2011; Scarpa et al., 2009; van Bokhoven et al., 2005b). It is important to replicate and extend these findings, preferably in longitudinal designs.

Because there are indications for complex interplays between neurobiological parameters and other, psychological and social, risk factors in relation to disruptive behavior (Raine, 2002b; van Goozen et al., 2007), such risk factors should be incorporated when studying the longitudinal relationships between ANS / HPA-axis parameters and disruptive behavior. It has been proposed in theoretical models that relationships between ANS / HPA-axis parameters and disruptive behavior are moderated by (early) adverse or traumatic experiences (Raine, 2002b; Susman, 2006; van Goozen et al., 2007), these experiences can lead to symptoms of posttraumatic stress disorder (PTSD) (Brosky & Lally, 2004). Furthermore, there are indications that the relation between experienced events and aggression is mediated by PTSD symptoms (Marsee, 2008; Ruchkin et al., 2007). Because PTSD symptoms have been related to altered stress (re)activity (Blechert et al., 2007; Kirsch et al., 2011; Pole, 2007) as well as to higher levels of (reactive) aggression (Kivisto et al., 2009; Marsee, 2008), it is expected that PTSD symptoms have a moderating effect as well. Because in delinquent high rates of PTSD symptoms were found, it is of particular importance to study this moderating effect in such samples (Colins et al., 2010; Marsee, 2008; Ruchkin et al., 2002; Vermeiren et al., 2006).

To address the above mentioned issues, the overall aim of this thesis was to investigate longitudinal relationships between ANS and HPA-axis activity and

reactivity, and specific types of disruptive behavior in delinquent male adolescents. In a prospective longitudinal design, delinquent male adolescents were assessed in early adolescence and after a follow-up period of five years. At baseline, 112 participants were included after referral by the police to a delinquency diversion program (age at baseline assessment: 12-14 years). Participants from the delinquent sample were divided in a subgroup with a disruptive behavior disorder (DP+) and a subgroup without such a disorder (DP-). As a control group, a normal adolescent sample was included (NC, n = 38), this sample was matched group-wise to the delinquent sample on age, IQ, SES, and ethnicity. Detailed information on the number of participants in each separate analysis is provided in chapter 1 and in the corresponding chapters of this thesis.

Both at baseline and at follow-up, participants and parents underwent behavioral assessment that included a structured psychiatric interview, as well as various questionnaires. At follow-up, the official police registration was used to obtain data on reoffending. Neurobiological assessment included heart rate, HRV, salivary cortisol and alpha-amylase, measured at rest and in response to a standardized psychosocial stress test procedure, consisting of a public speaking task (PST, Jansen et al., 2000). Cortisol was also assessed at home, directly after awaking and after 30 and 60 minutes, representing the Cortisol Awakening Response (CAR, Clow et al., 2004).

Five studies were conducted for the purpose of this thesis, specific aims and results are summarized, followed by a general discussion.

The aim of the study presented in **chapter 2** was to examine the predictive value of heart rate and HRV for reoffending in the delinquent sample. Data from the baseline assessment on heart rate and HRV were used, measured at rest and in response to the PST. Data on reoffending were obtained at follow-up, from the official Dutch police registration system for criminal behavior (*Herkenningsdienstsysteem, HKS*). Results showed that at follow-up, two thirds of the participants had reoffended. By means of survival analyses for recurrent events, it was found that attenuated heart rate reactivity significantly predicted a higher reoffending rate. Furthermore, we found that stronger HRV reactivity (increased vagal withdrawal) also predicted a higher reoffending rate. Notably, no predictive value of heart rate or HRV in resting conditions for reoffending was found. Although effect sizes were small to moderate, these findings underscore the consistency of the relationship between autonomic markers and antisocial behavior. The results provided evidence that reactivity of heart rate and HRV are neurobiological markers for persistent juvenile antisocial behavior.

The aim of the study presented in **chapter 3** was to examine the predictive

value of cortisol, heart rate and HRV for different types of disruptive behavior in the delinquent sample. As an extension to the registered reoffending in the previous chapter, the study in chapter 3 examined self-reported rule breaking behavior. Furthermore, self-reported overall aggression and proactive and reactive aggression were assessed, as well as categorical DBD diagnoses. In order to study the exclusive value of neurobiological parameters over and above disruptive behavior, levels of disruptive behavior measured at baseline were incorporated as well. Results showed that low resting HRV significantly predicted reactive aggression at follow-up, over and above baseline reactive aggression. Furthermore, there were significant interactions between neurobiological parameters and baseline disruptive behavior in relation to disruptive behavior at follow-up. These interactions showed that high resting heart rate and low resting HRV predicted aggressive behavior at follow-up, when baseline aggression was low. Attenuated heart rate responsivity predicted proactive aggression at follow-up, when baseline proactive aggression was high. Our findings provided evidence that neurobiological parameters have predictive value for juvenile disruptive behavior after 5-year follow-up, over and above baseline disruptive behavior. Furthermore, the results of this study indicate that distinctive neurobiological profiles may underlie reactive and proactive aggression.

One cross-sectional study in this thesis is presented in **chapter 4**. This study aimed to examine the additional value of alpha-amylase to cortisol, heart rate and HRV as potential correlate of juvenile disruptive behavior in delinquent male adolescents and controls. Furthermore, the combined activity as well as interactions between the various parameters in relation to disruptive behavior were investigated. Because alpha-amylase was only assessed at follow-up, this study has a cross-sectional design. Participants in this study were 48 delinquents and 16 normal controls who participated in the follow-up study. The mentioned neurobiological parameters were investigated at rest and in response to the public speaking task. A structured psychiatric interview (DISC) as well as the Youth Self Report (YSR) and Child Behavior Checklist (CBCL) were administered to assess disruptive behavior. Within the delinquent group, 15 participants had a DBD diagnoses (DP+), while 33 had not (DP-). None of the participants in the control group had a DBD diagnoses (NC). The results of this study showed that attenuated alpha-amylase and cortisol reactivity, but not heart rate or HRV, were significantly related to dimensional measures of disruptive behavior. Moreover, both cortisol and alpha-amylase reactivity were significantly lower in the DP+ group as compared to the NC group. Combining alpha-amylase and cortisol in one model explained a larger part of the variance of disruptive behavior than either single parameter. There were no interactions between alpha-amylase and cortisol

or HRV in relation to disruptive behavior. These results led us to the conclusion that attenuated alpha-amylase responsivity to stress is a correlate of disruptive behavior in late-adolescent males. Combining alpha-amylase and cortisol indeed improved insight into neurobiological mechanisms involved with disruptive behavior; concurrent low reactivity of both parameters was related to higher levels of disruptive behavior.

In **chapter 5**, a study is presented that aimed to investigate the stability of cortisol between baseline and follow-up assessment in two populations. For the purpose of this study, a large sample of adolescents from the general population was included (n = 231). This sample was derived from the TRAILS study (Tracking Adolescents' Individual Lives Survey, Huisman et al., 2008). Cortisol stability was assessed in the delinquent population as well as in the TRAILS sample. In both populations, cortisol samples were obtained directly after awakening and after 30 minutes, as representation of the cortisol awakening response (CAR). The CAR was obtained at baseline and after 5-year follow-up. For both populations applied that the cortisol samples at baseline and at follow-up were analyzed in different laboratories. Therefore, we could not investigate the stability of mean levels of cortisol. We were however able to investigate the coherence between the initial and follow-up measurements by means of correlations, taking into account the possible confounding influences of age, pubertal stage and smoking behavior. In both delinquent and general population samples, we did not find any significant correlations between cortisol measurements at baseline and follow-up assessments. Cortisol can thus not be regarded as a stable marker in adolescents, which has implications for the interpretation of research findings on cortisol measures and disruptive behavior.

The first aim of the study presented in **chapter 6** was to investigate stability of heart rate and HRV, as well as proactive and reactive aggression over five years in the delinquent sample. The second aim was to study longitudinal relationships between ANS parameters and proactive and reactive aggression, and, third, to investigate the moderating effect of PTSD symptoms on these relationships. At baseline and follow-up assessment, heart rate and HRV were measured at rest and in response to the PST. Furthermore, self-reported proactive and reactive aggression were assessed by means of the Reactive-Proactive aggression Questionnaire (RPQ). Results showed considerable stability of aggression and ANS parameters, except HRV reactivity. A longitudinal relationship was observed between attenuated HRV reactivity (decreased vagal withdrawal) and proactive but not reactive aggression. PTSD symptoms were found to be a moderator; the relationship between HRV reactivity and proactive aggression was present when levels of PTSD symptoms were low, whereas at high levels of PTSD symptoms this relationship diminished. This study provided further

indications for distinctive neurobiological profiles of proactive and reactive aggression. When studying associations between ANS parameters and aggression, it is important to incorporate the moderating influence of additional factors like PTSD symptoms.

GENERAL DISCUSSION

In the studies presented in this thesis longitudinal relationships between ANS and HPA-axis (re)activity and disruptive behavior were examined in delinquent male adolescents. Strengths of the studies were the incorporation of multiple neurobiological parameters as well as different types of disruptive behavior. Because we assessed the same group of participants twice, i.e. in early and late adolescence, our studies have provided new insights in several aspects of behavioral an neurobiological development throughout adolescence in delinquent males.

Disruptive behavior in our sample of delinquent male adolescents was found considerably stable after 5-year follow-up. Based on official police records, two thirds of the delinquents had reoffended during 5-year follow-up. Furthermore, half of the delinquents that showed a DBD at baseline, persisted in having this diagnosis, whereas the other half desisted. Levels of both proactive and reactive aggression were consistently high. These results show that boys who committed a minor offense in early adolescence show fairly high levels of disruptive behavior after 5-year follow-up, despite the referral to a delinquency diversion program. In general, although short-term effectiveness of intervention strategies for disruptive behavior (e.g. parent management training, cognitive behavioral therapy) has been demonstrated (Kazdin, 2000), the long-term effectiveness of treatment is considered to be limited (Fonagy et al., 2002). The results from our studies can be taken into account when developing additional, more specific, interventions.

An important overall result that emerged from our studies is that various types of disruptive behavior demonstrated differential neurobiological profiles. We found a predictive value of attenuated heart rate reactivity and increased heart rate variability (HRV) reactivity for the number of registered reoffenses committed during follow-up (chapter 2). High resting heart rate and low resting HRV were found to have a predictive value for overall aggression. With respect to subtypes of aggression, we found that low resting HRV had a predictive value for reactive aggression and that attenuated heart rate reactivity had a predictive value for proactive aggression. The latter result was extended in chapter 6, where we found a longitudinal relationship

between attenuated HRV reactivity and proactive aggression. Additional insight was provided by the results from the cross-sectional study, where attenuated alpha-amylase and cortisol reactivity were found to relate to DBD as well as externalizing behavior (chapter 4).

These observed differential relationships can be interpreted within some of the dominant theories on antisocial behavior. To date, most studies on disruptive behavior reflected on results on measures of general ANS, SNS and HPA-axis activity in the perspective of the low arousal theories, whereas results on PNS activity are viewed in the perspective of the polyvagal theory. Following this approach, we consider that our results on heart rate, cortisol and alpha-amylase reactivity fit in with the low arousal theory, and in particular the fearlessness theory. In this theory, low arousal is regarded as a marker of low levels of fear and low punishment sensitivity. Hence, fearless juveniles are thought to be more likely to engage in disruptive behaviors because they do not fear the negative consequences of their actions (fearlessness theory, Raine, 1993; Raine, 2002a). In line with this assumption, we found attenuated heart rate reactivity to relate to reoffending and proactive aggression (chapter 2 and 3), as well as attenuated cortisol and alpha-amylase reactivity relation to DBD and externalizing behavior (chapter 4). In contrast, our findings do not seem to support the sensation seeking theory. In this theory it is argued that low arousal represents an unpleasant physiological state that makes individuals seek out sensation to raise their arousal levels to an optimal or normal level (sensation seeking theory, Zuckerman, 1979). We did, however, not find associations between disruptive behavior and low baseline activity of heart rate, cortisol or alpha-amylase. Next, we consider that our findings on HRV fit in with the polyvagal theory. Within this theory, decreased parasympathetic activity or increased parasympathetic reactivity are regarded as a reflection of emotional dysregulation, which is a hallmark for psychopathology, including disruptive behavior (Beauchaine, 2001; Porges, 1995; Porges, 2007). In line with this theory, we found that low resting HRV and increased HRV reactivity were related to respectively reactive aggression and reoffending (chapter 2 and 3). Taken together, our results demonstrate that the two theories, low arousal and polyvagal, are not mutually exclusive and that it is useful to reflect on findings from the perspective of both theories. Furthermore, because differential neurobiological profiles may underlie specific subtypes of aggression, there is a need to further reflect on such specific behaviors within these theoretical models. Our results support this by showing attenuated heart rate reactivity (low arousal) to be related to proactive 'cold-blooded' aggression, and low HRV (emotional dysregulation) to be related to reactive 'emotional' aggression.

In our studies we showed that ANS / HPA-axis parameters have an additional value in explaining disruptive behavior, over and above well-known risk-factors of such behavior. One of these well-known risk-factors is the presence of disruptive behavior at baseline, which has been shown to predict disruptive behavior at follow-up (Burke et al., 2002; Loeber et al., 2009b). Our results revealed an exclusive predictive value of ANS parameters at baseline for aggressive behavior at follow-up, over and above disruptive behavior at baseline (chapter 3). Another risk-factor that we studied in combination with neurobiological parameters was the presence of PTSD symptoms. It has been argued in theoretical models that stress-related neurobiological systems like the ANS act as a mediator in the relation between (early) adverse or traumatic events and disruptive behavior (Raine, 2002b; van Goozen et al., 2007). Early adversities are proposed to play a role in the attenuation of such physiological systems, which in turn is considered an important mechanism involved in the development and persistence of disruptive behavior (Susman, 2006; van Goozen et al., 2007). We extended previous studies by studying PTSD symptoms, which can be the consequence of adverse or traumatic events. Our results on the moderating effect of PTSD symptoms showed that the longitudinal relationship between attenuated HRV and proactive aggression was only present at low levels of PTSD symptoms (chapter 6).

In addition to the advantage of combining neurobiological parameters with other risk-factors, the results of our studies highlight the benefit of combining information from parameters of several biological stress systems. We found that examining reactivity of both SNS and HPA-axis parameters in one model (respectively alpha-amylase and cortisol) explained a larger part of the variance of externalizing behavior than either single parameter alone (chapter 4). Our results provide support for a model proposed by Bauer (2002) suggesting that a disturbance in the interplay between different physiological stress-systems may predispose to disruptive behavior. Generally, both SNS and HPA-axis show increased reactivity in response to stress. Our results revealed that concurrent attenuated reactivity of alpha-amylase and cortisol was related to higher levels of externalizing behavior.

In our studies, different types of disruptive behavior showed differential neurobiological profiles, as discussed previously in this chapter. However, some measures of disruptive behavior did not show longitudinal relations with neurobiology. We did not find a predictive value of ANS or HPA-axis (re)activity for self-reported rule-breaking behavior (chapter 3). This difference as compared to our results on registered reoffending did not come as a surprise, because it is known that self-reported and registered (re)offending are only weakly correlated to one another (Maxfield et al.,

2000). Furthermore, official records reflect only part of all offenses committed by an individual, whereas serious / violent offenses tend to be underrepresented in self-reports (Maxfield et al., 2000), including the self-report used in chapter 3 (the rule-breaking scale of the YSR). It can thus be speculated that altered ANS reactivity is related to the more severe types of offenses, that were incorporated in the official report but not in our self-report questionnaire.

Another measure of disruptive behavior that was not predicted by either of the neurobiological parameters was a categorical DBD diagnosis at follow-up (chapter 3). An explanation for these absent longitudinal relationships, beside a general lack of power of categorical measures and the relatively small sample size, can be found in the heterogeneous nature of the DBD diagnosis; it comprises both ODD and CD. Because of our limited sample size, we did not further differentiate between these two separate diagnoses. However, even within both separate diagnoses, there is considerable heterogeneity. In fact, there is an ongoing debate regarding the DSM-V, to further subtype CD by incorporating callous-unemotional (CU) traits. CU traits have been associated with a fearless temperament and punishment insensitivity, and can identify juveniles at risk for severe and persistent delinquent behavior (Pardini & Fite, 2010). On the contrary, within ODD a subset of symptoms with irritable mood had been described, that are associated with later internalizing problems. This irritable mood and internalizing symptoms may result from a common underlying mechanism, i.e. problems with regulating negative emotions (Loeber et al., 2009a; Pardini & Fite, 2010). Future studies on neurobiological correlates of ODD and CD can consider to incorporate these additional features.

The results of our studies enhanced knowledge on stress-related neurobiological correlates of disruptive behavior, in addition to the existing psychological and social correlates. Nevertheless, in general, the effect sizes obtained in our studies were small, indicating that the neurobiological parameters predict a small part of the variance of disruptive behavior. Hence, the value of ANS / HPA-axis parameters for clinical practice is limited at this point. The neurobiological parameters can only be applied at the group level and they are not specific enough to be used as a diagnostic or screening tool in individuals. The biological values observed in the participants, whether it concerned increased or decreased (re)activity, were almost all within normal physiological ranges, and showed a large variance. More research is needed to assess the value of stress-related neurobiological parameters for clinical practice, see below.

Limitations

There are some methodological limitations of the study that should be considered when interpreting the results. First, we studied a specific population of delinquent male adolescents. Although studying such a specific group has evident relevance, results cannot be generalized to other samples like clinic-referred disruptive behavior disordered juveniles, very young offenders, or girls. With respect to the latter, in addition to the clear differences in the prevalence of antisocial behavior between boys and girls (Moffitt & Caspi, 2001), sex differences have been found in the relationships between ANS / HPA-axis activity and disruptive behavior (Beauchaine et al., 2008; Dietrich et al., 2007; Marsman et al., 2008). Whereas disruptive boys demonstrated reduced ANS / HPA-axis functioning, disruptive girls displayed increased HPA-axis activity and equal cardiovascular activity compared to controls. It has been suggested that girls' behaviors are being driven by different etiological mechanisms (Beauchaine et al., 2008). A second limitation is the size of our sample. The size of the total population was of adequate size, however, only a subsample was approached for performing the public speaking task. Furthermore, there was considerable attrition after 5-year follow-up, resulting in small sized groups for some of the analyses, for example the subgroups (DP+, DP-, NC) as studied in chapter 4. Although we found relevant significant results throughout the studies, the small sample size limited power to incorporate multiple neurobiological parameters combined with other correlates such as PTSD symptoms. Third, our measures concerning resting values were assessed prior to the public speaking task. Although participants were instructed to spend this time as relaxed as possible and they did not know the content of the task beforehand, neurobiological levels may have been influenced by anticipatory stress. Fourth, cortisol samples at baseline were analyzed in a different laboratory than the samples obtained at follow-up. Therefore, we could not investigate the stability of mean levels of cortisol, or could we perform formal longitudinal analyses on cortisol. We were however able to investigate the coherence between the initial and follow-up measurements by means of correlations.

Directions for future research

The results of our studies bring forward some recommendations for future research. Future longitudinal studies on relationships between neurobiological parameters and disruptive behavior are recommended to incorporate more than two assessments. Despite the longitudinal design of our study, we still cannot make statements on causality. With more assessments in larger samples, for example annually throughout adolescence, advanced statistical techniques such as time lag or structural equation

modeling can be conducted. This can provide more insight in causal relationships. Furthermore, in our studies we mainly used linear models to investigate relationships between neurobiological parameters and disruptive behavior. The small sample size prohibited us to compare subgroups within our sample longitudinally. Future studies can extend our findings, for example by comparing subgroups with persisting DBD to subgroups with desisting DBD.

In addition, an experimental procedure can be incorporated, for example a behavioral treatment or an induced change in ANS parameter with medication like methylphenidate. Assessing neurobiological and behavioral parameters before and after such an experiment can provide more insight in the co-occurrence of changes in neurobiological parameters and changes in disruptive behavior. Furthermore, such designs can help to predict effectiveness of current treatment options for juveniles with disruptive behavior. Preliminary evidence for this assumption was provided by two studies on school-aged children with DBD; one investigated resting heart rate (Stadler et al., 2008) and one investigated cortisol reactivity (van de Wiel et al., 2004). It was found that low heart rate and low cortisol reactivity predicted poor treatment outcome, as compared to high heart rate or high cortisol reactivity. When these results can be extended in other samples and with other (combinations of) neurobiological parameters, specific neurobiological profiles may be used in the future as an additional tool to allocate the most optimal treatment to juveniles with disruptive behavior.

Our result that alpha-amylase responsivity to stress is a correlate of disruptive behavior in late adolescent males needs replication in other samples. Furthermore, longitudinal designs are needed to study the dynamics of this relation over time. One study by Keller (2009) revealed a curvilinear relationship between alpha-amylase in 9-year old children and externalizing behavior after 2-year follow-up. It was shown that children at age 9 with either low or high levels of resting alpha-amylase activity showed highest levels of externalizing at age 11. Children with moderate alpha-amylase levels at age 9 showed lowest levels of externalizing behavior at age 11 (Keller & El-Sheikh, 2009). When studying alpha-amylase, it is advised to take into recommendations and considerations regarding sampling procedures provided by Rohleder (2009), Nater (2009) and Bosch (2011).

With respect to cortisol, future longitudinal studies have to take into account that analyzing cortisol samples in different batches can lead to differences in mean values and ranges. The differences in laboratories between the first and second assessment precluded us to perform formal longitudinal analyses on cortisol. Nevertheless, both at baseline and at follow-up assessment we found attenuated cortisol reactivity in

the DP+ group compared to the NC group. Because the composition of the DP+ group at follow-up was different compared to baseline, it can be hypothesized that a change in cortisol reactivity is related to a change in disruptive behavior. Furthermore, persistent attenuated cortisol reactivity may relate to persistent DBD. Future longitudinal studies incorporating repeatedly measured cortisol can provide additional insight on this.

The neurobiological parameters that were studied are all easily accessible peripheral parameters, reflecting the end products of different autonomic and neuroendocrine cascades, starting in the brain. With respect to the central coordination of the stress response, both the ANS and the HPA-axis are guided by a complex network of brain regions including the amygdala, orbital frontal cortex, anterior cingulate cortex, insular cortex, and other interconnected regions (Dolan, 2002). A disruption in the central regulation of the stress response may be an important correlate of juvenile disruptive behavior (Bauer et al., 2002). Results from brain imaging studies indeed showed evidence for functional and structural abnormalities in these regions in disruptive juveniles (Stadler et al., 2010). Future studies are needed to acquire a further understanding on the physiological mechanisms by which these parameters are related to disruptive behavior.

One result that emerged from our studies is the advantage of concurrently assessing neurobiological parameters from different stress-regulating systems. Besides measuring parameters from multiple neurobiological systems concurrently, future studies can combine basal activity and stress reactivity within models. It has recently been shown that focusing on levels of basal activity along with the response to stress has also potential to provide additional information (El-Sheikh & Hinnant, 2011).

With respect to non-biological correlates of disruptive behavior, we showed the moderating influence of PTSD symptoms. Besides the influence of early adversities or PTSD symptoms, there appear to be complex interplays between neurobiological parameters and psychological and social factors. For example, although resting heart rate is often found to be lower in antisocial individuals, it may be a particularly strong characteristic of antisocial individuals from higher social classes (Raine & Venables, 1984). Moreover, the presence of both biological and social risk factors appears to exponentially increase rates of antisocial and violent behavior (Raine, 2002b). When studying different neurobiological, psychological and social factors in large populations, it can be studied which combination of factors is best predictive of future and persistent disruptive behavior. Comprehensive theoretical models, for example as provided by Van Goozen (2007), can provide a framework from which

such studies can be performed. This may guide to further increasing our knowledge and understanding of disruptive behavior. Ultimately, prevention and intervention strategies for juveniles who show disruptive behavior and who are at risk for a deviant development can be optimized.