Examining physiological stress (re)activity as an endophenotype for adolescent substance use

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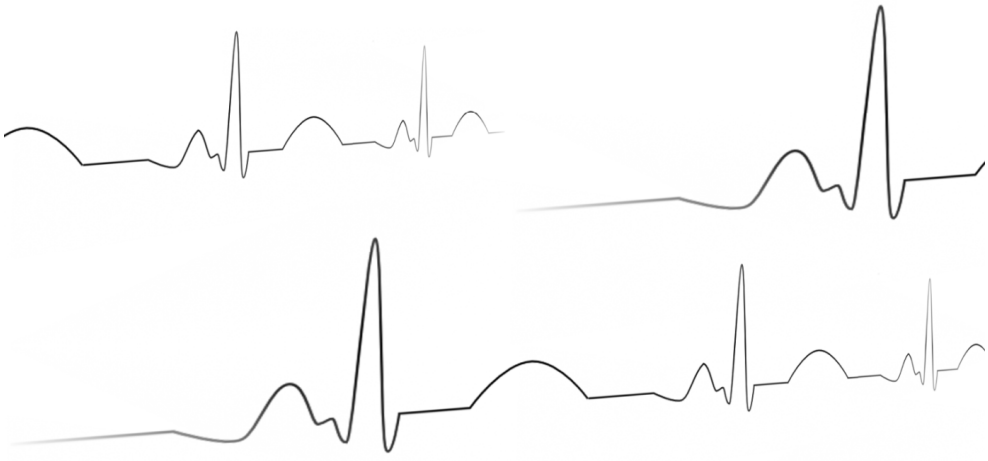
2013

document version
Publisher's PDF, also known as Version of record

Chapter 7

General discussion



Chapter 7

The aim of the present thesis was to investigate physiological stress (re)activity as an endophenotype for substance use in adolescents. Dysregulated physiological stress (re)activity has been observed in animals with a heightened tendency to engage in substance abusing behavior (Kosten & Ambrosio, 2002; Richardson, et al., 2008) and in patients with substance use disorders (SUDs; Sinha, 2008). However, it is unclear from these findings whether such dysregulation is the consequence of chronic and heavy substance use or whether it is an underlying vulnerability. Therefore, we examined physiological stress (re)activity in individuals at risk for the development of SUDs. Specifically, we examined indices of the autonomic nervous system (ANS, i.e. heart rate and respiratory sinhus arrhythmia; RSA) and hypothalamic-pituitary-adrenal (HPA) axis (i.e. cortisol) (re)activity during a psychosocial stress procedure in adolescents from the general population who portrayed varying levels of risky substance use and in the children of parents with a SUD (CPSUDs). Furthermore, we examined potential determinants of physiological stress reactivity and the relation between perceived and physiological stress reactivity in children and adolescents from the general population.

We first discuss the results of physiological stress reactivity in relation to risk for SUDs (chapters three through six). Subsequently, we discuss the findings from chapter two and integrate the findings from all of the studies.

PHYSIOLOGICAL STRESS (RE)ACTIVITY IN RELATION TO SUBSTANCE USE DISORDERS

Physiological stress (re)activity in adolescents from the general population

Risky substance use (i.e. early onset or frequent use of substances) during adolescence strongly predicts an increased chance of developing substance use problems later in life (DeWit, et al., 2000; Grant & Dawson, 1997; Gruber, et al., 1996; Hawkins, et al., 1997; Sung, et al., 2004). Therefore, adolescents who exhibit risky substance use behavior are at heightened risk for the development of SUDs, and therefore may be more likely to portray vulnerability markers for SUDs than adolescents who do not exhibit this behavior. In the third and fourth chapters of this thesis, we examined adolescent risky substance use in relation to cortisol and heart rate

(re)activity during a psychosocial stress procedure. We found that adolescents who began drinking at an earlier age showed lower cortisol levels at the onset of and during the stressful tasks, but not during the recovery period (chapter three). Diurnal cortisol levels and the cortisol awakening response were not related to age of onset of alcohol use (chapter three).

In chapter four, we observed lower mean heart rates during the entire psychosocial stress procedure in adolescents who reported consuming a medium and high number of drinks per week (more than two) compared to adolescents who consumed two drinks or less per week. Furthermore, adolescents who used tobacco every day portrayed blunted heart rate reactivity compared to adolescents who smoked less frequently or not at all. These findings, coupled with the results from earlier studies in adolescents from the general population (i.e. Huizink, et al., 2006; van Leeuwen, et al., 2011), suggest physiological hypo-arousal during psychosocial stress in adolescents prone to risky substance use. Interestingly, in our studies, frequency of alcohol use was related to mean heart rate, but not cortisol levels. Age of onset of alcohol use, on the other hand, was related to cortisol levels. The reason for this is not entirely clear, however, as both frequent and early onset of alcohol use during adolescence are considered risky substance use behavior, we believe that the findings from both studies suggest physiological hypo-arousal in adolescents who are prone to risky substance use.

Physiological stress (re)activity in adolescents with familial risk for substance use disorders

Because CPSUDs are at increased risk for developing SUDs themselves later in life (Bohman, 1978; Chassin, et al., 1991; Cloninger, et al., 1981; Goodwin, et al., 1973; Johnson & Leff, 1999; Kendler, et al., 2008; Tessner & Hill, 2010), these individuals, like adolescent risky substance users, are expected to be more likely to manifest endophenotypes for SUDs than adolescents whose parents have not been diagnosed with a SUD. In the fifth and sixth chapters of this thesis, we examined diurnal and stress-evoked cortisol levels and ANS (re)activity during a psychosocial stressor in CPSUDs and controls. We did not observe any differences between CPSUDs and controls in diurnal cortisol levels, but CPSUDs showed blunted cortisol levels in anticipation of the stressor (chapter six), which is in line with earlier work done by Moss and colleagues (i.e. Dawes, et al., 1999; Hardie, et al., 2002; Moss, et al., 1999; Moss, et al., 1995). Unlike two studies in adult CPSUDs (Dai, et al., 2002; Sorocco, et al., 2006), we did not observe

blunted cortisol levels in response to the psychosocial stressor. In chapter five, similar to previous findings, we observed blunted heart rate recovery in CPSUDs as compared to controls, and increased resting RSA levels. Blunted heart rate recovery also partially mediated the relation between CPSUDs reporting more externalizing behaviors, and being more likely to use tobacco daily.

Thus, our findings from chapters three through six, in combination with findings from previous research (e.g. Huizink, et al., 2006; Moss, et al., 1995) suggest physiological hypo-arousal in adolescents vulnerable to SUDs. These individuals may be inherently hypo-aroused, which leads them to actively seek out substances in order to achieve physiological comfort (Goeders, 2003; Majewska, 2002). This active seeking of substances may be related to a more general personality-related tendency to seek out stimulation (i.e. stimulation-seeking hypothesis; Creemers, et al., 2009; Martin, et al., 2002; Zuckerman & Kuhlman, 2000). On the other hand, it may be related to the lack of a physiological 'brake' when confronted with dangerous or prohibited activities, thus increasing the chance of engaging in delinquent, externalizing or risky substance use behaviors (Raine, 1993).

PHYSIOLOGICAL STRESS (RE)ACTIVITY

Determinants of physiological stress reactivity

In recent years, physiological stress reactivity has been increasingly investigated as an endophenotype for (mental) health problems. However, there is no consensus in the literature as to what factors interfere with physiological responding. In chapter two of this thesis, we aimed to explore determinants of physiological stress reactivity in a stratified sample of children (aged 7-12 years) and adolescents (aged 13-20 years). We examined the influence of individual, developmental, environmental and substance-use related factors on four indices of stress reactivity. We observed different determinants for RSA, heart rate, cortisol and perceived stress reactivity, and different determinants in children versus adolescents. Cortisol reactivity was related to sex and perceived parental emotional warmth in children, and emotionality, sociability, urbanicity, and parental involvement in adolescents. Heart rate reactivity was related to

urbanicity and socioeconomic status in both children and adolescents, and furthermore sex, sociability, parental involvement and tobacco use in adolescents. RSA reactivity was related to age and socioeconomic status in children, and activity level in adolescents. Perceived stress reactivity was related to shyness, age and perceived parental rejection in children, and perceived parental overprotection, parent-reported inconsistent discipline and tobacco use in adolescents. From these results, it became clear that individual (especially sex and temperamental factors), developmental (especially age in children) environmental (especially related to living conditions and parenting factors), and substance use-related (especially tobacco use) factors have significant influences on physiological stress reactivity. Studies increasingly examine physiological stress reactivity as a vulnerability marker for (mental) health problems. This study showed that it is imperative that such studies take into consideration determinants of physiological stress reactivity that may account for found relations. We provided an indication of which determinants should be considered in studies in children and adolescents.

Age effects

Perhaps the most intriguing finding in chapter two was the difference in the stress reactivity patterns in children as compared to adolescents. Children showed the expected pattern of increasing cortisol levels and decreasing RSA levels during the stressful tasks as compared to the pre-task rest period. Adolescents, however, did not show this pattern. Cortisol levels were highest and RSA levels were lowest at the beginning of the stress procedure, and did not increase/decrease further during the tasks. This indicates a clear difference in physiological stress reactivity patterns for cortisol and RSA levels between children and adolescents. Mean heart rate and perceived stress levels showed similar patterns during the psychosocial stress procedure in both samples. Animal studies showed dramatic differences in the physiological stress systems in adolescents compared to adults (Romeo, 2010), but more research on the development of these systems is necessary to understand their role as endophenotypes for (mental) health problems.

Perceived stress

It is often postulated that perceived stress reactivity mirrors physiological stress reactivity (e.g. Thayer, 1970). However, convincing evidence of this is minimal (i.e. Oldehinkel, et al., 2011). In the second chapter of this thesis, we examined this issue in children and adolescents separately.

We found that perceived stress reactivity predicted cortisol reactivity in adolescents only, but none of the other physiological stress responses in children or adolescents, although these relations were marginally significant ($.05 < p < .12$; and reached significance when children and adolescents were pooled). We thus conclude that the relation between perceived and physiological stress reactivity may be weakly but positively related, and likely to be detectable only in large samples, as in the Oldehinkel study (Oldehinkel, et al., 2011) and ours when children and adolescents were pooled.

Few studies have examined perceived stress reactivity in groups at risk for SUDs. We observed no differences in perceived stress in adolescents who began drinking at different ages (chapter three), however, we did find that perceived stress ratings at the onset of the psychosocial stress procedure were lower in CPSUDs as compared to controls. This was an interesting finding, and in contrast to earlier studies which found higher perceived stress during the stressor (Uhart, et al., 2006; Zimmermann, et al., 2004) and no differences (Finn & Pihl, 1987; Sorocco, et al., 2006) in CPSUDs when compared to controls. At this point, it is not entirely clear what the relation between perceived and physiological stress reactivity is in CPSUDs compared to controls.

Diurnal cortisol levels

In this thesis, we found no differences in diurnal cortisol levels in adolescents who began drinking at an earlier age as compared to those who began drinking at a later age (chapter three), and in CPSUDs as compared to controls (chapter six). These results confirmed similar earlier studies (Dai, et al., 2002; Gianoulakis, et al., 2003; Hernandez-Avila, et al., 2002; Huizink, et al., 2009; Waltman, et al., 1994; Wand, et al., 2001; Wand, et al., 1999). In one study, though, the cortisol awakening response was found to be blunted in early onset cannabis users (Huizink, et al., 2006). However, in light of several studies that did not observe such differences, we suggest that diurnal cortisol levels may not be dysregulated in adolescents who are vulnerable to SUDs. Cortisol levels in anticipation of or during psychosocial stress may more likely explain physiological differences between adolescents at heightened risk for SUDs and those who are not.

Physiological responding: anticipation, reactivity and recovery

In both of our studies that investigated cortisol levels in relation to vulnerability to SUDs, the strongest effects were found at the beginning of the psychosocial stress procedure. Cortisol levels were highest at onset of the procedure and did not increase further, or only minimally, during the stressful tasks. Indeed, anticipation effects at the beginning of a stress procedure are known to blunt subsequent cortisol responses (Nicolson, 2008). To examine whether cortisol levels were higher at the beginning of the stress procedure as compared to resting cortisol levels, we compared cortisol levels at onset of the psychosocial stress procedure to those taken on a normal day in the afternoon (comparable to the time that the psychosocial stress procedure began). In chapter three, cortisol levels were observably higher in moderate, late and never drinkers at the beginning of the procedure as compared to the afternoon cortisol measurement taken on a normal day. Early drinkers, however, showed comparatively lower cortisol levels. Similarly, in chapter six, controls had higher cortisol levels at the beginning of the procedure compared to on a normal day, while CPSUDs showed lower cortisol levels. Other studies have reported the strongest effects in anticipation of a stressor in contrast to during it (e.g. Ellis, et al., 2005; Sumter, et al., 2010; Westenberg, et al., 2009), thus our findings were not entirely surprising. Furthermore, our findings are in line with those from Moss and colleagues of higher cortisol levels in adolescent male CPSUDs in anticipation of a novel situation as compared to controls (Dawes, et al., 1999; Hardie, et al., 2002; Moss, et al., 1999; Moss, et al., 1995).

One of the unexpected and interesting findings from chapter two was that children and adolescents differed in their patterns of cortisol and RSA reactivity. Cortisol and RSA patterns showed strong anticipation effects in adolescents, whereas in children this was not the case. Considering that the studies in chapters three and six were performed in adolescents (aged 14-20 and 11-20, respectively), it seems reasonable that the strongest effects of age of onset of drinking or of familial risk for SUDs were found in anticipation of stress, as opposed to during the stressful tasks. Possibly, high cortisol levels in anticipation of psychosocial stress form the normative adolescent HPA axis response to stress, perhaps indicating cognitive preparation for an upcoming stressor, and could be related to executive functions (i.e. planning, cognitive flexibility) which develop increasingly during adolescence (Davidson, et al., 2006). Dysregulation of this response may denote developmental delays in cognitive and/or

physiological functioning, and limited ability to prepare for an upcoming stressor in adolescents at risk for SUDs.

In chapter six, the cortisol reactivity slope was significantly positive across groups, indicating a significant increase in cortisol levels in response to the stressful tasks, although this increase was minimal. Other studies have similarly found increasing cortisol levels in response to tasks in adolescents (e.g. Gunnar, et al., 2009; Prince van Leeuwen, et al., 2011). Most of these utilized a lengthy pre-task adaptation period (30-60 min), which is important in order to allow cortisol levels to decrease to resting levels prior to stressful tasks (Gunnar, et al., 2009). In chapter three, cortisol levels in adolescents did not increase further in response to the stressful tasks. Our pre-task session, during which participants filled in questionnaires and then sat quietly for ten minutes, was approximately 20 minutes long. This appears to have been sufficient time for children's cortisol levels to decrease substantially, however, adolescents may have stronger anticipatory responses, and therefore may need more time before cortisol levels approach resting levels.

Reactivity measures are often calculated as the difference between the pre-task rest and stressful tasks, although some studies have used the post-task rest as a 'baseline' measure due to anticipation effects found at the onset of the stressor (e.g. Hansen, Johnsen, & Thayer, 2003; Oldehinkel, et al., 2011). Recovery is much less often studied, though it may be regulated through different mechanisms than reactivity (Kamarck & Lovallo, 2003), and may be an important additional measure (Linden, et al., 1997). The ability to recover from stress may be a critical part of being able to adapt to the environment and return to pre-stress arousal levels, and therefore, this measure has high ecological validity. In our studies, we examined both reactivity and recovery measures. In chapter three, age of onset of drinking alcohol did not predict differences in cortisol recovery. In chapter five, however, differences between CPSUDs and controls were strongest for heart rate recovery, not reactivity. In light of these findings, we recommend future research should take care to examine physiological recovery in addition to reactivity.

Hypo-activity versus hypo-reactivity

In this thesis, our findings provide evidence for both physiological hypo-activity (i.e. general arousal level) and hypo-*re*activity (i.e. in response to a task). In the studies on cortisol levels (chapters three and six), our findings suggest hypo-activity, because cortisol levels were lower at the beginning of the stress procedure, but cortisol reactivity did not differ between risk groups. Similar findings were observed in chapter four for the relation between mean heart rate during the psychosocial stress procedure and number of drinks consumed per week. Adolescents who drank more than two drinks per week exhibited lower mean heart rates during the stressor, but showed a similar heart rate response to the tasks compared to adolescents who drank less. In the same study, though, adolescents who used tobacco daily showed blunted heart rate *reactivity* to the tasks. Similarly, in chapter five, blunted heart rate reactivity and recovery were related to experiencing more externalizing symptoms, and a higher likelihood of using tobacco daily in both CPSUDs and controls. Furthermore, CPSUDs showed blunted heart rate recovery as compared to controls. Physiological hyp*er*-(re)activity has been previously reported in patients (e.g. Gerra, et al., 2008) and CPSUDs (e.g. Harden & Pihl, 1995), however, we did not find evidence for this in any of our studies. All in all, our studies suggest both hypo-activity (observed mostly in cortisol levels) and hypo-reactivity (observed in mean heart rate in response to stress). Physiological dysregulation thus seems to be evident in the form of hypo-arousal in adolescents at heightened risk for SUDs later in life.

Calculating stress reactivity

There are several ways of calculating physiological stress reactivity. Multilevel modeling is the most advanced method to analyze physiological data at this time, and the most efficient. Advantages of this method are outlined in Adam and Gunnar (2001). In short, within-person correlation between physiological measurements are accounted for on the first level, and between-person predictors are examined on the second level. If sufficient variance is observed in physiological measurements, this warrants the examination of between-individual predictors of these physiological measurements. Moreover, multilevel modeling utilizes all data points, thereby efficiently handling missing data. We employed this method for cortisol measurements in the sixth chapter. Other methods are frequently used and can have the advantage of being

simpler and in this way are more parsimonious. For cortisol measurements, area under the curve calculations are recommended (Pruessner, et al., 2003), and can be calculated with respect to ground, as we did for diurnal cortisol levels in the third chapter, or with respect to increase, which provides a measure of cortisol reactivity. Like this method, repeated measures analyses of variance utilizes all data points, which provides more information on the pattern of the stress response, but neither of these techniques handles missing data as effectively as multilevel modeling. In some situations, as in chapters two and five of this thesis, reactivity and recovery measures provide sufficient information to answer the research question. Like area under the curve calculations, these measures are continuous variables and can thus be entered into regression models as predictors or outcomes.

SUBSTANCE USE AS AN EXTERNALIZING DISORDER

Physiological hypo-arousal is observed in adolescents prone to risky substance use as well as in adolescents who exhibit more externalizing behaviors (Ortiz & Raine, 2004). Therefore, physiological hypo-arousal could be an endophenotype for externalizing behaviors in general or for substance use specifically. In order to shed more light on this matter, we examined the relation between adolescent risky substance use and heart rate (re)activity during psychosocial stress while controlling for externalizing symptoms (chapter four). We found that the relation between adolescent risky substance use and lower mean heart rates during the stressor was not attenuated when externalizing behaviors was entered into the model. Similarly, in chapter five, we examined the influence of familial risk for SUDs and ANS (re)activity on the outcomes of externalizing behaviors and substance use simultaneously (multivariate model). The relations of familial risk status and ANS (re)activity remained significant to both externalizing behaviors and tobacco use. The findings from both of these studies suggest that, while physiological hypo-arousal is related to externalizing behaviors (chapter five), this does not account for the relations between physiological hypo-arousal and substance use. Therefore, we conclude that physiological hypo-arousal can be viewed as an endophenotype for both substance use and externalizing behaviors, independently of one another. Although, due to strong comorbidity

between these phenotypes (e.g. Zimmermann, et al., 2007), they are most likely not completely independent.

MECHANISMS EXPLAINING THE RELATION BETWEEN PHYSIOLOGICAL DYSREGULATION AND RISK FOR SUBSTANCE USE DISORDERS

Given the findings described in this thesis and in previous research showing that physiological hypo-arousal may be associated with a vulnerability to SUDs, we now address potential mechanisms that underlie this association. For one, it is most likely that genetics play a role. Several genes, such as the brain derived neurotropic factor (BDNF), the dopamine receptor (D2), the muscarinic acetylcholine receptor (CHRM2), and catechol-o-methyltransferase (COMT) have been related to both physiological stress (re)activity and vulnerability to SUDs (Colzato, van der Does, Kouwenhoven, Elzinga, & Hommel, 2011; Edenberg, et al., 2004; Fisher, Vincent, Gomeza, Yamada, & Wess, 2004; Gerra, et al., 2003; Mueller, et al., 2012; Wang, et al., 2001). Although some specific effects are known, much research is still needed in this area, as well as integrating epigenetic processes in order to understand the role of genetics in the association between vulnerability to SUDs and physiological stress (re)activity.

Another factor that could be of influence is the effect of prenatal exposure to substances. Substance use in pregnant women is known to affect the developing HPA axis of the fetus (Huizink & Mulder, 2006). Several studies have provided evidence for cortisol dysregulation in infants, children and adolescents who were exposed to intrauterine substances (Bauer, et al., 2011; Chaplin, et al., 2010; Fisher, et al., 2012; Hunter, et al., 2011; Lester, et al., 2010). In our studies, though, less than three percent of mothers in the general population sample and less than nine percent of mothers in the CPSUD sample reported using substances during pregnancy. We therefore expect that this does not explain the relation of physiological hypo-arousal and vulnerability to SUDs in our samples. A related potential mechanism is prenatal exposure to high levels of maternal stress, which is also known to affect the developing HPA axis of the fetus (Huizink, et al., 2004; Kudielka & Wüst, 2010). We do not have data available in our studies on maternal stress during pregnancy, and therefore cannot examine or rule out this influence.

A commonly cited finding in the literature is that life stressors lead to subsequent blunted physiological responses in adults (e.g. Lovallo, et al., 2012) and in children (e.g. Gunnar & Vazquez, 2001). It is plausible that adolescents who are more likely to exhibit risky substance use have experienced more stressful life events. Likewise, CPSUDs are known to experience greater lifetime adversity than their peers who do not have a parent with a SUD (Hussong, et al., 2008). Therefore, the physiological hypo-arousal observed in CPSUDs could be the result of having experienced more adverse life events. We tested this hypothesis in chapter six, but found no evidence to support it. CPSUDs showed lower cortisol levels at onset of the stress procedure, but this was not related to having experienced more adverse life events, although CPSUDs did report having experienced significantly more such events than controls. Perhaps one reason why we did not find this expected association is that it may not be so much general adverse life events that are related to hypo-arousal in CPSUDs, but specifically parental abuse and neglect, which are experienced substantially more often in CPSUDs (Ammerman, et al., 1999). This could also very well account for the findings in our studies in adolescents from the general population. As we do not have information available on parental abuse and neglect specifically, future studies will be necessary in order to examine this possible mechanism.

A final potential mechanism is that adolescent substance use has directly affected the physiological stress systems. Our studies are cross-sectional in nature, and therefore we cannot exclude this possibility. Studies have showed that alcohol use may influence the HPA axis directly (Richardson, et al., 2008). In the fifth chapter, CPSUDs reported being more likely to use cannabis, and were marginally more likely to use tobacco daily. Our findings from chapters three and four, moreover, indicated physiological hypo-arousal in adolescents more likely to begin drinking at an earlier age, more likely to drink a higher number of drinks per week, and more likely to use tobacco daily. Thus, it is possible that the physiological hypo-arousal observed in our studies was directly due to substance use. Although we cannot exclude this possibility, we consider it unlikely due to the fact that the adolescents participating in our general population study, because of their young age, are not likely to have used substances heavily or chronically, and therefore, the substance use may not have been sufficient to affect physiological responding.

STRENGTHS AND LIMITATIONS

This thesis should be considered in light of some limitations. Firstly, the study was performed cross-sectionally, and therefore we cannot exclude with certainty the possibility that adolescent substance use directly affected physiological stress (re)activity. While we consider this unlikely, due to the fact that adolescents have generally not used substances as heavily or for as long as adult heavy substance users and SUD patients have, future studies, in younger children, and with a longitudinal design, will be necessary in order to rule out this possibility definitely. Secondly, it would have been preferable to have more information regarding the parents, especially in the CPSUD sample. More particularly, we were unable to take into account how long the parent had been struggling with the SUD, how long he/she had been in treatment and whether and for how long he/she was abstinent. Furthermore, in the CPSUD sample but also in the general population sample, we had limited information on parental psychopathology. Because of this, we are unable to conclude whether the physiological hypo-arousal observed indicates vulnerability to SUDs specifically or psychopathology in general. Thirdly, considering the emphasis on stress reactivity as an endophenotype in this thesis, it would have been pertinent to include genetic data. In this way, the pathway from genes to physiological stress (re)activity to substance use in adolescents could be examined. Unfortunately, this was not feasible at the current stage of the project, however, we hope to investigate this in the future.

However, despite these limitations, our studies contained a number of noteworthy strengths. For one, we were able to examine a relatively large general population sample of children and adolescents. Seven hundred eleven individuals participated in the psychosocial stress procedure, which provided data with sufficient variability in physiological stress (re)activity to be able to examine determinants of stress reactivity, the relation to perceived stress, and the relation to risky substance use. Also, we were able to recruit a relatively large sample of CPSUDs (83 individuals), mostly from outpatient addiction clinics. Because of this relatively large sample, we had sufficient statistical power to detect (even small) differences in physiological stress (re)activity. Furthermore, we were able to obtain comprehensive measures of physiological stress (re)activity. To examine HPA axis (re)activity, we utilized four measurements on a normal day, in order to track the diurnal rhythm of cortisol levels, as well as six measurements during a psychosocial stress procedure. Heart rate was assessed continuously

during the stressor, and because our stressor included tasks during which the participant experienced stress but was not speaking, we were able to assess RSA during rest as well as during psychosocial stress.

IMPLICATIONS

The findings in this thesis suggest an underlying physiological under-arousal in adolescents who are at heightened risk for SUDs later in life. Here we outline several implications of these findings for practice and research.

One possibility for intervention is biofeedback training. Heart rate variability training is currently used for treatment of asthma and hypertension, and since recently has been extended to the treatment of depression and anxiety (Lehrer, 2007). In this kind of training, feedback is provided through visual or auditory signals that are extracted from a physiological recording device. Through this feedback, one learns how to increase RSA. Heart rate increases during inhalation and decreases during exhalation. These changes in heart rate are RSA, which helps to control the ANS. By learning to increase these changes, one is better able to handle everyday stress (Lehrer, 2007). This method targets conditions characterized by low vagal tone, as emotion dysregulation is proposed to be (El-Sheikh & Erath, 2011; Porges, 2007), and it is therefore possible that the method could be used in adolescents with externalizing symptoms, and perhaps risky substance use as well. Biofeedback training has been suggested by Raine for adolescents who exhibit physiological hypo-arousal (Raine & Liu, 1998).

A related possibility for strengthening the ANS is physical exercise. Repeated activation and recovery of the system, through physical exertion, could help to stabilize the ANS and enhance adaptive responding. Research has shown that regular physical exercise leads to beneficial effects on ANS functioning (Felber Dietrich, et al., 2008). It is also used to treat symptoms of depression (Rimer, et al., 2012), however the mechanism behind this is not yet known. One intervention study in adolescent girls with depressive symptoms showed fewer symptoms and reduced physiological resting levels (urinary cortisol and heart rate) in those who had been assigned to an eight-week exercise regime as compared to those who had continued

their usual daily activities (Nabkasorn, et al., 2006). Possibly, this method could be extended to adolescents who show physiological hypo-arousal. If physical exercise could help the ANS to respond adaptively, such adolescents may be better equipped to inhibit stimulus-seeking or externalizing behavior, and thus perhaps help prevent SUD development.

Our studies underscore previous research findings that CPSUDs are at risk for negative outcomes. In our studies, CPSUDs reported experiencing significantly more adverse life events, reported significantly more internalizing and externalizing symptoms, reported perceiving significantly more parental rejection, were more likely to have used cannabis, and were marginally more likely to use tobacco daily. Because of this, it is important to monitor these children from a young age in order to be able to signal problems that may develop at an early stage. A difficult issue is how to identify CPSUDs, as many parents do not enter treatment, or do so after many years of struggling with a SUD, and when in treatment, may not be willing to address issues concerning their children. Therefore, it may be more appropriate to target children, for example through social workers at schools. Children could be targeted broadly, by raising awareness of and addressing openly parental substance abuse and dependence, and be alerted to ways in which children can ask for help themselves in dealing with such issues. In this way, the child's needs and problems can be addressed specifically, without necessary involvement of the parent.

The findings from chapter two provide practical implications for future studies examining physiological stress reactivity as a vulnerability marker for (mental) health problems. We systematically tested potential determinants of physiological stress reactivity which were individual, developmental, environmental and substance use-related in nature. We observed different determinants for RSA, heart rate, cortisol and perceived stress reactivity, and this differed between children and adolescents. Thus, we were able to clarify the extent to which the physiological variable is influenced by other factors. In considering these physiological variables as endophenotypes for health problems, future studies will be able to refer to our findings. In the same chapter we examined whether perceived stress reactivity was related to physiological stress reactivity and found that perceived stress reactivity significantly predicted cortisol reactivity in adolescents only, but none of the other measures of physiological reactivity in our stratified sample of children and adolescents. When children and adolescents were pooled, though, these

relations were all significant and positive. Therefore, future researchers should be aware that, though the relation between perceived and physiological stress reactivity is positive, it is clearly a weak relation, and it should not be assumed that these constructs are suggestive of the same underlying dimension.

Due in part to the findings in chapter two of cortisol and RSA reactivity being divergent in adolescents as compared to children, we realize there is insufficient fundamental knowledge on the development of physiological stress (re)activity from late childhood through adolescence. Previous research in animals showed dramatic differences in physiological reactivity in adolescence as compared to adulthood (Romeo, 2010), and some studies in humans showed differences between adolescents of different ages (Gunnar, et al., 2009; Hollenstein, et al., 2012; Stroud, et al., 2009), but much more research is needed in order to examine specifically the effects of age, puberty, sex, hormonal changes and cognition on physiological stress (re)activity during this transitional period.

More research is also necessary to elucidate the influence of different types of stressors. For example, daily stressors (such as school work), though mild, may have a considerable impact on physiological stress. Because adolescence is a period of social, environmental and biological changes, such stressors may be particularly salient during this period (Dahl & Spear, 2004). Experiencing severe stressors such as trauma has been shown to be consequential for physiological stress (re)activity (e.g. Heim, et al., 2000), however, too little is known about factors regarding the stressor and the individual experiencing it. For instance, the duration of the stressor could be of influence, i.e. whether it is chronic such as parental abuse or neglect, or relatively brief, such as being robbed, or periodic, as parental episodes of depression or SUD can be. The timing of such events may also be important, as children and adolescents could be particularly vulnerable to the impact of these events during certain developmental windows. Other influences on physiological stress (re)activity, such as socioeconomic status or urbanicity, as we showed in chapter two, may be chronic for a certain period of time, but then diminish, if the family moves to a different neighborhood for instance. It is important to know if the experienced stressor influences the developing stress system enduringly or whether adjustment is possible, and whether there is a specific period before which such adjustment should occur, such

as adolescence. Furthermore, more information is needed on how children and adolescents perceive stressors, and the influence this has, if any, on physiological stress (re)activity.

Pertaining to vulnerability to SUDs, more research is needed in the field of genetics in order to gain insight into the influences of specific genes and gene processes. Though complex, this research, coupled with research on endophenotypes, will help pinpoint more precisely individuals with heightened risk for SUDs, and perhaps distinguish individuals who are more likely to respond to different treatments. Information is also needed on whether physiological hypo-arousal in CPSUDs indicates a vulnerability to SUDs specifically or to psychopathology in general. In our studies, many SUD patients were also diagnosed with other psychiatric disorders such as attention deficit hyperactive disorder and depression, as is common. In future studies, it would be ideal to include children of parents with SUDs and varying levels of other psychiatric disorders in order to examine this matter more precisely.

In studying vulnerability to SUDs, it is crucial to keep in mind that the development of SUDs depends on several factors other than familial risk. For instance, substance use initiation and maintenance are influenced by personality (Creemers, Verhulst, & Huizink, 2009), peer (Geels, Vink, van Beijsterveldt, Bartels, & Boomsma, 2013; Korhonen, et al., 2008) and family (Swadi, 1999) factors and adverse life events (Conner, Hellemann, Ritchie, & Noble, 2010), to name only a few. Many CPSUDs, though statistically at a higher risk for developing SUDs themselves, are highly resilient and may not experience more life stressors or develop symptoms of psychopathology (Johnson & Leff, 1999). Future research should thus also focus on resilience factors, such as social support, personality factors or coping mechanisms, in combination with vulnerability factors. Another important addition to this field of research would be to examine CPSUDs at a younger age, for example during late childhood, before they begin using substances themselves. In this way, it could be excluded with certainty whether physiological hypo-arousal is a consequence of substance use or not. Ideally, research on vulnerability to SUDs should begin prenatally, whereby the individual is followed and examined intermittently throughout development. Such studies could begin in addiction clinics, or could be community-based (such as the Dutch Generation R cohort study), following a large number of individuals, some of whom will be more vulnerable to developing SUDs.

To conclude, research since the early 20th century, in different disciplines, has formed the foundation on which the studies in this thesis are based. Initial studies indicated the negative consequences of substance use problems for individuals and their children (Keynes, 1911; van den Brink, 2009). Adoption studies in the 1970s provided evidence for a clear genetic component of SUDs (Bohman, 1978; Goodwin, et al., 1973), but due to the complexity of genetics, research has more recently turned to neurobiological models, including the study of endophenotypes. Grounded in the work of Seyle and Cannon (Cannon, 1929; Seyle, 1950), physiological stress (re)activity has been investigated as an endophenotype for SUDs. Research in both animals and humans indicated a strong association between stress and the development of substance use behaviors (e.g. Richardson, et al., 2008; Sinha, 2008). Clinical studies confirmed dysregulated physiological stress (re)activity in patients with SUDs (e.g. Goeders, 2003; Lovallo, 2011), however, it cannot be discerned from this research whether such dysregulation is an underlying mechanism or a consequence of chronic and heavy substance use. In order to shed more light on this manner, we examined physiological stress (re)activity in individuals who are considered to be at risk for SUDs, that is, adolescents from the general population who exhibit risky substance use behaviors and the (adolescent) offspring of patients with SUDs. In general, the results of four studies in these two samples indicated physiological hypo-arousal during a psychosocial stressor in adolescents who are vulnerable to developing SUDs. These individuals may be inherently hypo-aroused, which may lead them to actively seek out substances in order to achieve a more comfortable level of arousal. Thus, this thesis provides beginning evidence for physiological stress (re)activity as an endophenotype for substance use in adolescents. Due to the cross-sectional nature of our studies, future research with a longitudinal design is necessary to confirm our findings. This thesis provides a keystone for such studies, and an initial impetus for research on the prevention of SUDs.