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

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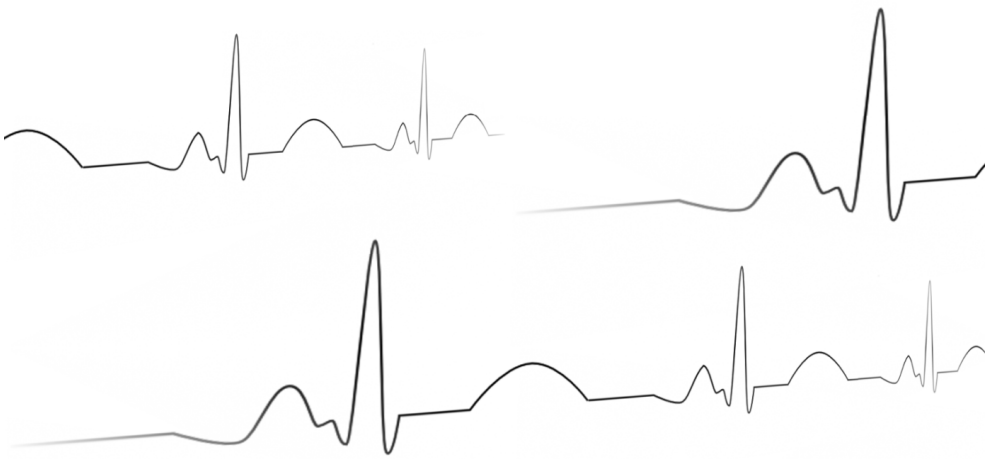
Chapter 1

General introduction



Chapter 1

BACKGROUND

Substance use has been a part of human culture for at least the past six thousand years; the earliest records indicate the use of alcohol in Egyptian and Mesopotomian cultures and opium in Neolithic Switzerland (Durrant & Thakker, 2003). Later records show the use of cannabis in China and coca leaves in South America a few thousand years ago (Nelson, 2012), the use of wine in classical Greek culture, tobacco in the pre-Colombus Americas, and cannabis during the middle ages in Europe (Durrant & Thakker, 2003). In these earlier times, substances were mostly used for pragmatic, medicinal or religious purposes (Durrant & Thakker, 2003; Nelson, 2012).

It was not until the late 17th century that the potentially dangerous health consequences of substance use began to be publically discussed, beginning with John Jones's book *Mysteries of Opium Reveal'd* (Durrant & Thakker, 2003), and strongly reinforced a century later by Dr. Benjamin Rush, who initiated the first rehabilitation clinics for alcoholics (Gifford, Friedman, & Majerus, 2010). During this time, addiction, or what we now term substance use disorders (SUDs; APA, 2000), was generally viewed as a consequence of moral weakness. During the 19th century, attention turned to the inescapable pharmacological effects of substances, and the best remedy for SUDs seemed to be to prevent use of substances. This idea led in part to the *prohibition* of alcohol in the United States in the 1920s and early 1930s (van den Brink, 2009). It became clear, though, that substances did not affect all individuals in the same manner, and SUDs began to be viewed as a symptom of an underlying personality disorder (van den Brink, 2009). Starting in the 1940s, the disease model became prevalent (Mold, 2004), whereby a combination of biological and psychological characteristics were thought to underlie vulnerability to SUDs. After that, ideas shifted towards a learning model, in which SUDs were seen as non-adaptive learned behaviors (van den Brink, 2009). In the 1970s, two adoption studies established a clear genetic component of SUDs (Bohman, 1978; Goodwin, Schulsinger, Hermansen, Guze, & Winokur, 1973), and these findings became integrated with the ideas of the previous models to form the biopsychosocial developmental model. This model emphasized the continuous interplay between genes, learning experiences and social environment in the development and maintenance of SUDs (van den Brink, 2009).

In recent years, the biological aspect of the biopsychosocial model, including genetics, has become increasingly important. However, the influence of specific genes is marginal and both genetic influence and phenotypes are highly variable between persons (Dick, Prescott, & McGue, 2009). Furthermore, gene-gene interactions, and gene-environment correlations and interactions are critical elements that need to be disentangled (Kendler, et al., 2012). Due in part to this complexity, neurobiological models have become prevalent, and researchers have turned to examining endophenotypes, also referred to as intermediate phenotypes, biomarkers or vulnerability markers, to explain the biological underpinnings of SUDs (Gottesman & Gould, 2003). Endophenotypes are subclinical traits that are related to a disorder and are thought to indicate genetic susceptibility for the disorder in non-affected individuals (Leboyer, et al., 1998).

One such potential endophenotype for SUDs is physiological stress (re)activity. Initial research in animals and humans indicated a strong association between stress and the development of substance dependent behaviors (Richardson, Lee, O'Dell, Koob, & Rivier, 2008; Sinha, 2008). Dysregulated physiological reactivity to stress may indicate a tendency to self-medicate in order to alleviate feelings of depression or anxiety (inherent *hyper*-arousal; Khantzian, 2003) or the tendency to actively seek out stimulation in order to achieve a more normative level of arousal (inherent *hypo*-arousal; Goeders, 2003; Majewska, 2002). Following research in animals, clinical research in humans confirmed aberrant physiological responding in abstinent patients with SUDs (for reviews, see Goeders, 2003; Lovallo, 2011; Sinha, 2008), including evidence for both hyper- and hypo-arousal. A clear disadvantage of clinical research among SUD patient populations, however, is that it does not resolve whether dysregulated physiological reactivity is an underlying mechanism or a consequence of chronic and heavy substance use.

Studies in individuals that are known to be at risk for SUDs, without having used substances heavily themselves, are therefore imperative to research on the influence of physiological stress (re)activity on the development of SUDs. One such population consists of adolescents who use substances frequently and/or begin using at an early age; early-onset and frequent substance use during adolescence are strong predictors of SUDs later in life, and as such, may be regarded as risky substance use (Bonomo, Bowes, Coffey, Carlin, & Patton, 2004; DeWit, Adlaf, Offord, & Ogborne, 2000; Grant & Dawson, 1997; Gruber, DiClemente,

Anderson, & Lodico, 1996; Hawkins, et al., 1997; Sung, Erkanli, Angold, & Costello, 2004). Another population consists of the offspring of patients with SUDs. These individuals are likewise known to be at risk for developing SUDs later in life, as demonstrated in twin, adoption and general population studies (Bohman, 1978; Chassin, Rogosch, & Barrera, 1991; Cloninger, Bohman, & Sigvardson, 1981; Goodwin, et al., 1973; Johnson & Leff, 1999; Kendler, Schmitt, Aggen, & Prescott, 2008; Tessner & Hill, 2010). In this thesis, we aimed to explore physiological stress (re)activity as an endophenotype for substance use in adolescents. To this end, we included adolescents from the general population as well as adolescents with familial risk for SUDs (i.e. the children of parents with a SUD).

PHYSIOLOGICAL STRESS (RE)ACTIVITY

The pioneering work of physiologists Hans Seyle (Seyle, 1950) and Walter Cannon (Cannon, 1929) was key in introducing the idea that the stress response is adaptive. Seyle saw stress as the adaptive, nonspecific response of the body to any demand (Seyle, 1976). Later researchers, beginning with Mason (Mason, 1968a, 1968b), emphasized the specificity of physiological processes to distinct emotional states.

Here we first give a general background of the physiological stress systems. There are two main systems: the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. In general, these systems can be examined at rest, in response to a stressor (reactivity) and following a stressor (recovery).

Autonomic nervous system

The ANS is made up of two main branches: the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The vagus, or 10^{th} cranial nerve, is a primary component of the PNS that innervates the sinoatrial node of the heart. According to polyvagal theory, the phylogenetically newer, myelinated, ventral branch of the vagus, often referred to as the vagal brake, is responsible for maintaining homeostasis and supporting social engagement during rest (Porges, 1995; Porges, 2007). The vagus maintains a low heart rate, constricted bronchi, contracted bladder, and activation of gastrointestinal functions. When an organism is confronted

with a stressor, the most immediate response involves disengagement of the vagal brake, or vagal withdrawal, indicating the organism's preparedness to respond to an anticipated stressor. Respiratory-sinus-arrhythmia (RSA) and heart rate variability are both frequently assessed and considered valid indices of vagal tone (Porges, 1995). If the response of vagal withdrawal is insufficient, the older SNS is activated, entailing the fight-or-flight response which elevates heart rate, increases blood pressure, dilates the pupils, inhibits saliva flow, dilates the bronchi, increases secretion of adrenaline and noradrenaline, suppresses gastrointestinal functions, and inhibits bladder contraction. The oldest part of the ANS involves the unmeyelinated, dorsal branch of the vagus, which gives rise to the freeze response (Porges, 1995; Porges, 2007). After the stressor subsides, SNS activity declines and vagal tone increases to return to resting levels. The heart is innervated by both the PNS and SNS. As described in the autonomic space model, the PNS and SNS operate independently of one another, and usually exert reciprocal influences on the heart (Berntson, Cacioppo, & Quigley, 1991). Thus, vagal withdrawal is followed by SNS activation, leading to an increase in heart rate in response to a stressor.

Hypothalamic-pituitary-adrenal axis

The HPA axis, like the ANS, is a phylogenetically ancient system, with elements of the system present in not only vertebrates but also invertebrates (Boyce & Ellis, 2005). The HPA response entails the production of corticotropin-releasing hormone by neurons in the paraventricular nucleus of the hypothalamus. This stimulates the secretion of adrenocorticotrophic hormone in the pituitary which in turn stimulates the secretion of cortisol in the outer cortex of the adrenal gland. As the cortisol in saliva is unbound and biologically active (Tornhage, 2009), salivary cortisol is often used in research because of its methodological facileness for participants. On normal days, the cortisol levels of healthy individuals follow a diurnal curve with an acrophase approximately 30 minutes after awakening and a subsequent gradual decrease towards an evening nadir (Schmidt-Reinwald, et al., 1999). The morning peak in cortisol is known as the cortisol awakening response and reflects (re)activity of the HPA axis (Wust, Wolf, Hellhammer, & Kirschbaum, 2001). When confronted with a stressful situation, the adaptive response of a healthy individual is a temporary increase in the secretion of cortisol and a successive decline in cortisol levels after the stressor no longer poses a threat (e.g. Sapolsky, Romero, & Munck, 2000).

Chapter 1

Physiological response to psychosocial stress

Both the ANS and HPA axis respond to endogenous and exogenous stressors, including psychological stress (Munck, Guyre & Holbrook, 1984; Lovallo, 2005). The ANS responds quickly to stressors, while the HPA axis responds much slower, and also serves to inhibit activation of the ANS, preventing pathological over-activation (Sapolsky, et al., 2000). In the field of SUD research, assessing physiological stress reactivity to a psychosocial stressor is preferable due to its ecological validity, as this is what is most likely to be encountered in daily life. The Trier Social Stress Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) is a valid and widely used task to induce both ANS and HPA axis responding (Kirschbaum, 2010). Participants are asked to perform a mental arithmetic task and a personal speech in front of judges and/or a camera, thereby provoking psychosocial stress. The most important elements of the task are uncontrollability and social-evaluative threat (Dickerson & Kemeny, 2004). In this thesis, the psychosocial stress procedure was modeled closely after the TSST.

Aberrant stress physiology

In the field of psychophysiology, where physiological stress responding is related to behavioral outcomes, it is important to understand what entails healthy (and unhealthy) physiological responding. Researchers often examine one or more indices of the ANS and/or HPA axis. In the present thesis, RSA, heart rate and cortisol were examined, therefore, the majority of the literature review pertains to these indices.

At rest, higher vagal tone is proposed to be healthy as it promotes social engagement behavior (Porges, 2007), keeping resting heart rate at a minimum. Optimal responding entails rapid, pronounced vagal withdrawal (i.e. a decrease in RSA), and following the stressor, a rapid and pronounced return to higher vagal levels (Porges, 1995; Porges, 2007). Differences in resting vagal tone and vagal withdrawal are thought to indicate individual differences in emotionality and emotion regulation, respectively (Beauchaine, 2001; Porges, 2007). Hence, low resting vagal tone and reduced withdrawal may signify emotion dysregulation (El-Sheikh & Erath, 2011; Porges, 2007). Weak RSA recovery may indicate maladaptive emotional responding (Santucci, et al., 2008).

Heart rate and cortisol levels are generally low during rest. During stress, heart rate increases quickly and, subsequent to the stressor, decreases quickly, usually within a few minutes (Hugdahl, 1995; Turner, 1994). Physiological recovery may be regulated through different mechanisms than reactivity (Kamarck & Lovallo, 2003), and delayed recovery may be critical in signaling aberrations of normal regulatory processes (Steptoe, 2007). Cortisol levels rise in response to the stressor, observable in saliva approximately 20 minutes after the onset of the stressor, and decline slowly again following the stressor (Sapolsky, et al., 2000).

Low resting heart rate and reduced heart rate reactivity are thought to indicate fearlessness, and thereby reduced inhibition to engaging in delinquent or antisocial behavior (Raine, 1993). Moreover, blunted heart rate recovery has been reported to indicate general health risks (e.g. Cole, Blackstone, Pashkow, Snader, & Lauer, 1999; Georgoulias, et al., 2003). A second hypothesis draws on the observation that individuals with high sensation seeking tendencies are more likely to engage in substance use (Creemers et al., 2009; Martin et al., 2002; Zuckerman and Kuhlman, 2000). These individuals may be inherently hypo-aroused and deliberately seek out substances in order to achieve a state of normalized arousal and thereby physiological comfort (Goeders, 2003; Majewska, 2002), also referred to as the stimulus-seeking hypothesis (Huizink et al., 2006; Zuckerman and Neeb, 1979). Both of these theories propose that physiological *hypo*-arousal is aberrant, while the self-medication theory proposes that inherent physiological *hyper*-arousal indicates a vulnerability to psychopathology (Khatzian, 1985). Both views may prove to be valid, as has been postulated using rodent models; rats strains that are inherently hypo-aroused as well as those that are hyper-aroused show a greater tendency towards substance abusing behaviors compared to rats that are not inherently hypo- or hyper-aroused (Kosten & Ambrosio, 2002).

Determinants of physiological and perceived stress reactivity

Since the 1930s, stress has been recognized to play an essential role in the development and maintenance of diseases and (mental) health problems (Seyle, 1976). However, it has only been relatively recently that physiological stress (re)activity has been investigated as an endophenotype on the pathway between genes and health outcomes. The past decades have shown a significant increase in studies relating aberrant physiological stress reactivity to negative

health outcomes such as cardiovascular diseases (Lovallo, 2011), anxiety and depressive disorders (Greaves-Lord, et al., 2007; Kagan, Reznick, & Snidman, 1987) and disruptive behavioral disorders (Ortiz & Raine, 2004) to name only a few. Despite the growth of research in this area, there is still very little fundamental knowledge about physiological stress reactivity in children and adolescents. A number of theories (i.e. Alpert & Wilson, 1992; Lazarus & Folkman, 1984; McEwen, 1998) do outline several proposed determinants of physiological stress reactivity, which are individual (i.e. sex), developmental (i.e. age), environmental (i.e. socioeconomic status) and substance use-related (i.e. caffeine intake) in nature. Though reviews are available on potential determinants, especially concerning the HPA axis (Jessop & Turner-Cobb, 2008; Kirschbaum & Hellhammer, 1989; Kudielka, Hellhammer, & Wüst, 2009; Kudielka & Wüst, 2010), few studies have systematically tested such determinants in one study. Therefore, in the second chapter of this thesis, we addressed this gap in the literature by examining determinants of RSA, heart rate, cortisol and perceived stress reactivity in a stratified sample of children and adolescents from the general population.

Perceived stress reactivity

Physiological stress reactivity is generally postulated to be reflected in the perceived physiological response to stress (i.e. the subjective perception of physiological arousal; Thayer, 1970). However, empirical examinations of this relation have yielded inconclusive findings (e.g. Cohen, et al., 2000; Dickerson & Kemeny, 2004; Hjortskov, Garde, Orbaek, & Hansen, 2004; Schlotz, Hammerfald, Ehlert, & Gaabb, 2011; Thayer, 1970). A recent study examined this question in a large sample of adolescents from the general population. Although effect sizes were small, a positive association between physiological and perceived stress reactivity was found (Oldehinkel, et al., 2011). Conclusive evidence is still needed to elucidate the relation between physiological and perceived stress measures in response to a psychosocial stressor across a wider age range. Therefore, we examined this relation in children and adolescents, spanning the ages 7 to 20 years, in the second chapter of this thesis.

Given the central focus in this thesis on the relation between risk for SUDs and physiological stress, it may be interesting to examine, albeit exploratively, the relation between risk for SUDs and perceived stress. If adolescents at risk for SUDs perceive the stressor

differently than controls, this could indicate differences in the subjective experience of stressors encountered in daily life. In the few studies that have examined this relation, the results have been equivocal. Two studies reported higher perceived stress reactivity during a stressor in individuals with familial risk for SUDs as compared to controls (Uhart, Oswald, McCaul, Chong, & Wand, 2006; Zimmermann, et al., 2004), while others reported no differences between groups (Finn & Pihl, 1987; Sorocco, Lovallo, Vincent, & Collins, 2006). In order to shed more light on this matter, we examined differences in perceived stress reactivity in adolescents from the general population who varied in current frequency of alcohol and tobacco use (chapter four) and in the children of patients with SUDs as compared to controls (chapter six).

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

Physiological stress responding is a viable endophenotype for SUDs for several reasons. Firstly, ANS and HPA response patterns are partially genetically determined (Bartels, van den Berg, Sluyter, Boomsma, & de Geus, 2003; Federenko, Nagamine, Hellhammer, Wadhwa, & Wüst, 2004; Mueller, et al., 2012; Wüst, et al., 2004) and have been shown to be relatively stable individual characteristics from childhood on (Berntson, Cacioppo, & Quigley, 1993; Carroll, Turner, Lee, & Stephenson, 1984; Harshfield, et al., 1999; Robinson, Whitsett, & Kaplan, 1987). Secondly, initial studies indicated that these response systems may be dysregulated in SUD patients, adolescent risky substance users and individuals with familial risk for SUDs (i.e. the children of patients with SUDs).

Physiological stress (re)activity in patient populations

A relatively large body of literature on physiological stress (re)activity in SUD patients and heavy drinkers suggests both ANS and HPA axis dysregulation in these individuals. Resting cortisol levels may be higher in SUD patients compared to controls (Gerra, et al., 2003; Gerra, et al., 2008; Sinha, et al., 2009) and in heavy drinkers compared to light or non-drinkers (Boschloo, et al., 2011; Gianoulakis, Dai, & Brown, 2003; Thayer, Hall, Sollers, & Fischer, 2006). Similar results were found in chronic smokers compared to non-smokers, although this could be due to

acute effects of repeated nicotine intake (Richards, et al., 2011). One study found no differences between SUD patients and controls in the cortisol diurnal pattern (Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000). In response to stress, most studies reported HPA axis hypo-reactivity in SUD patients compared to controls (Errico, Parsons, King, & Lovallo, 1993; Gerra, et al., 2003; Lovallo, et al., 2000; Sinha, et al., 2009), although one study did not find such differences (Munro, Oswald, Weerts, McCaul, & Wand, 2005). Similar findings were reported for smokers compared to non-smokers (Richards, et al., 2011), though in one study only in women (Back, et al., 2008).

Pertaining to ANS (re)activity, most studies reported higher resting mean heart rate in alcohol dependent patients compared to controls (Ingjaldsson, Laberg, & Thayer, 2003; Sinha, et al., 2009), in heavy drinkers compared to moderate drinkers (but not compared to non-drinkers; Boschloo, et al., 2011), and in smokers compared to non-smokers (al'Absi, Wittmers, Erickson, Hatsukami, & Crouse, 2003; Phillips, Der, Hunt, & Carroll, 2009; Richards, et al., 2011; Sheffield, Smith, Carroll, Shipley, & Marmot, 1997; Tsuda, Steptoe, West, Fieldman, & Kirschbaum, 1996). In contrast, one study suggested that smokers may not differ from non-smokers on resting RSA (Richards, et al., 2011). Heart rate hypo-reactivity is mostly reported in response to stress in SUD patients versus controls (Panknin, Dickensheets, Nixon, & Lovallo, 2002), and in smokers compared to non-smokers (Girdler, Jamner, Jarvik, Soles, & Shapiro, 1997; Richards, et al., 2011; Sheffield, et al., 1997; Straneva, Hinderliter, Wells, Lenahan, & Girdler, 2000), in both light and heavy smokers (Phillips, et al., 2009; Roy, Steptoe, & Kirschbaum, 1994). RSA hypo-reactivity is likewise reported in SUD patients compared to controls (Ingjaldsson, et al., 2003; Thayer, et al., 2006) and in smokers compared to non-smokers (Richards, et al., 2011). Taken together, most of these findings suggest elevated resting cortisol levels and mean heart rate and physiological hypo-reactivity to stress. However, from these clinical studies, it is not clear whether physiological dysregulation is the consequence of chronic and heavy substance use.

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

In order to gain more insight into physiological stress (re)activity as an endophenotype for substance use, that is, physiological dysregulation *not* due to the direct use of substances,

researchers have examined adolescents from the general population who show varying levels of risky substance use. Results from such studies point toward HPA axis dysregulation in adolescents who began using substances early or frequently. These studies were performed within the TRAILS (Tracking Adolescents' Individual Lives Survey) general population study, which followed a large group of youth longitudinally through adolescence. The first study observed that adolescents who began using cannabis at an early age (9-12 years) had an attenuated cortisol awakening response at the time of the first measurement (age 10-12) as compared to adolescents who reported first use of cannabis at a later age (Huizink, Ferdinand, Ormel, & Verhulst, 2006). In a follow-up study, present and future tobacco smoking was related to increased cortisol levels in response to awakening, but present and future alcohol use were unrelated to cortisol levels (Huizink, Greaves-Lord, Oldehinkel, Ormel, & Verhulst, 2009). A third study in a focus group examined cortisol levels in response to psychosocial stress. Adolescents who had used cannabis showed lower cortisol reactivity levels than those who had not used cannabis and who had only used tobacco. Furthermore, repeated cannabis users showed blunted reactivity compared to adolescents who had used cannabis or tobacco only a few times (Prince van Leeuwen, et al., 2011).

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

Children of parents with a SUD (CPSUDs) are known to be at increased risk for developing substance use problems 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). Therefore, studies in these individuals also help clarify the relation between physiological stress (re)activity and vulnerability to SUDs. In the late 1980s researchers had begun to investigate dysregulation of the physiological stress systems as an endophenotype for SUDs in CPSUDs. Pertaining to the ANS, heart rate hyper-reactivity in response to unavoidable shock was reported in adult men (Finn & Pihl, 1987; Pihl, Peterson, & Finn, 1990). In response to psychosocial stress, heart rate hypo-reactivity was found in adults (Sorocco, et al., 2006), but hyper-reactivity was found in adolescents (Harden & Pihl, 1995). Only one study investigated RSA activity, and found that children with parents who indicated problem drinking did not differ from controls (resting and reactivity; El-Sheikh, 2001), although low resting RSA has been observed in youth

exposed to frequent familial conflict (El-Sheikh & Erath, 2011), which is postulated to be more prevalent in families with a parent with a SUD (Johnson & Leff, 1999).

Regarding HPA activity, several studies examined acute cortisol responses to alcohol versus placebo in CPSUDs as compared to controls. While the cortisol response to alcohol is not relevant to the present thesis, the placebo conditions provide an indication of resting cortisol levels. The majority of studies reported no differences between adult CPSUDs and controls in resting cortisol levels (Dai, Thavundayil, & Gianoulakis, 2002; Gianoulakis, et al., 2003; Hernandez-Avila, Oncken, Van Kirk, Wand, & Kranzler, 2002; Waltman, McCaul, & Wand, 1994; Wand, McCaul, Gotjen, Reynolds, & Lee, 2001; Wand, Mangold, Ali, & Giggey, 1999), or in diurnal cortisol curve patterns (Gianoulakis, Dai, Thavundayil, & Brown, 2005; Sorocco, et al., 2006; Wand, et al., 1999). However, differences between CPSUDs' and controls' HPA axis response to stress have been reported. In adolescents and adults, most studies observed lower cortisol levels in CPSUDs as compared to controls in anticipation of a novel situation (Dawes, et al., 1999; Hardie, Moss, Vanyukov, Yao, & Kirillovac, 2002; Moss, Vanyukov, Yao, & Kirillova, 1999; Moss, Vanyukov, & Martin, 1995), and in response to psychosocial stress (Dai, et al., 2002; Sorocco, et al., 2006). However, in two studies HPA axis hyper-activity was observed in adult CPSUDs in response to psychosocial stress, although only in European-Americans (Uhart, et al., 2006), and only if the placebo session was administered first (as opposed to the alcohol administration session; Zimmermann, et al., 2004).

Most of the studies mentioned above concern either adults or young adolescents, and most included men only. Furthermore, the studies varied considerably with regard to the type of stressor used and the sample size, and only one investigated RSA responses in CPSUDs. To shed more light on this matter, in the present thesis, we examined HPA axis and ANS (re)activity during psychosocial stress in a relatively large sample of adolescent CPSUDs and controls.

SUBSTANCE USE AS AN EXTERNALIZING BEHAVIOR

Substance use can be viewed as a manifestation of child and adolescent behavioral (externalizing) problems (symptoms of e.g. oppositional defiant disorder, conduct disorder;

Krueger, et al., 2002; Liu, Raine, Wuerker, Venables, & Mednick, 2009). The relation between physiological stress (re)activity and externalizing problems has been reasonably well established. In general, low resting physiological activity and hypo-reactivity to stress have been observed; as evidenced in investigations of RSA (El-Sheikh & Erath, 2011; Porges, 2007), mean heart rate (Fairchild, et al., 2008; Hastings, Zahn-Waxler, & Usher, 2007; Ortiz & Raine, 2004; Popma, et al., 2006; Van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & Van Engeland, 2000) and cortisol (Pajer, Gardner, Kirillova, & Vanyukov, 2001; Snoek, Van Goozen, Matthys, Buitelaar, & Van Engeland, 2004; Van Goozen, et al., 1998). However, a few studies did not observe such associations (Alink, et al., 2008; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Willemen, Schuengel, & Koot, 2009), or reported *hyper*-reactivity in those with more symptoms of externalizing problems (Lorber, 2004; Van Goozen, et al., 1998).

Because physiological hypo-arousal may be expected in adolescents at risk for SUDs and is found in adolescents who report more externalizing behaviors than healthy adolescents, it is of interest whether physiological hypo-arousal could be viewed as an endophenotype for SUDs specifically, or for externalizing behaviors in general. In order to examine this, in chapter four, we controlled for externalizing behaviors when examining the relation between adolescent risky substance users and heart rate (re)activity. Furthermore, in chapter five, we examined externalizing behaviors and substance use as outcome variables in multivariate models. In these models, we examined whether familial risk for SUDs and ANS (re)activity predicted externalizing behaviors and risky substance use.

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

In this thesis, we hypothesize physiological dysregulation in adolescents who are at risk for developing SUDs later in life. What could be potential mechanisms underlying such a relation? Genes are certain to play an important role, heritability estimates for SUDs being relatively high (between 40-70%; Kendler, et al., 2012). We expect that the CPSUDs in our sample are genetically at high risk for developing SUDs because they are the offspring of patients with SUDs. Physiological responding has also been shown to be partially genetically regulated (i.e.

Mueller, et al., 2012), and therefore physiological dysregulation may be genetically inherited by the offspring of patients with SUDs. However, an alternative explanation is possible. CPSUDs are frequently reported to have experienced greater lifetime adversity than their peers who do not have a parent with a SUD (Hussong, et al., 2008). CPSUDs are met with greater family-related environmental adversity (Johnson & Leff, 1999), and are more likely to be physically and sexually abused (Miller, Maguin, & Downs, 1997). Furthermore, experiencing stressful life events has been reported to lead to subsequent blunted physiological reactivity in adults (e.g. Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012) and in children (e.g. Gunnar & Vazquez, 2001). Therefore, it is plausible that physiological dysregulation found in CPSUDs may be the consequence of having experienced more lifetime adversity. In chapter six, we examined this possibility by testing whether having experienced more adverse life events affected the relation between familial risk for SUDs and cortisol levels during a psychosocial stress procedure.

AIMS

The aim of the present thesis was to examine physiological stress (re)activity as a potential endophenotype for substance use in adolescents. In a sample of children and adolescents from the general population and a high risk sample of CPSUDs, we measured cortisol levels, RSA and mean heart rate during a psychosocial stress procedure. Outcome measures consisted of substance use and externalizing behaviors. In chapter two, we aimed to investigate the nature of physiological stress reactivity on a more general level, specifically, we examined factors that could be considered determinants of physiological stress reactivity in children and adolescents. In this study, we also examined the relation between perceived and physiological stress reactivity. The specific aims of the present thesis were to examine:

1. Determinants of perceived and physiological stress reactivity (**chapter two**);

2. The relation between perceived and physiological stress reactivity (**chapter two**);

3. The relation between HPA axis activity and age of onset of alcohol use in adolescents (**chapter three**);

4. The relation between ANS activity and frequency of alcohol and tobacco use in adolescents (**chapter four**);

4a. The relation between perceived stress reactivity and frequency of alcohol and tobacco use in adolescents (**chapter four**);

5. The relation between familial risk for SUDs, ANS (re)activity and externalizing behaviors and substance use (**chapter five**);

6. Whether cortisol levels during a psychosocial stress procedure differed between CPSUDs and controls (**chapter six**);

6a. Whether perceived stress during the psychosocial stress procedure differed between CPSUDs and controls (**chapter six**);

7. Whether experiencing more adverse life events could account for differences in cortisol levels between CPSUDs and controls (**chapter six**).

SAMPLES AND PROCEDURE

The participants in the studies in this thesis were part of the JOiN (Jongeren Onderzoek in Nederland; Youth Research in the Netherlands) study, which consisted of a sample of children and adolescents randomly drawn from the general population living in the province of South Holland, the Netherlands, and a sample of CPSUDs, recruited mainly through their parents who were in treatment for a SUD at the Bouman GGZ clinics in the same area. Details of the sample and design of the study were published in Huizink, et al. (2012).

General population sample

Participants in the general population sample were part of an ongoing longitudinal study that aimed to examine the development of emotional and behavioral problems in children and adolescents (Tick, van der Ende, & Verhulst, 2007). At the first assessment wave (T1), 2286 eligible children and adolescents were randomly drawn from the registers of 35 municipalities. Of these, 1710 individuals participated in T1. At T2, 1161 of the participants fulfilled inclusion criteria, of whom 990 participated. The T2 measurement consisted of questionnaires as well as a psychosocial stress procedure, which took place between November 2004 and March 2009.

Seven hundred and eleven children and adolescents between the ages of 7 and 20 years participated in this stress procedure.

Children of parents with a substance use disorder

The sample of CPSUDs consisted of 83 adolescents between the ages of 11 and 20 who had at least one parent who had been diagnosed with a SUD (lifetime DSM-IV diagnosis of substance abuse and/or dependence other than nicotine). Adolescents participated in the study between June 2009 and December 2011. The majority of these adolescents were recruited from outpatient clinics of Bouman GGZ, the major addiction care provider in South Holland, where their parents were in treatment for a SUD. Diagnosis of SUD in parents was based on clinical consensus obtained by clinical staff at Bouman GGZ based upon extensive observation and interviewing of the clients. A few adolescents (n=6) were recruited through the Bouman GGZ Youth Clinic, being in treatment themselves, and their parents were known to have a diagnosis of SUD. A number of participants (n=6) had parents who were diagnosed with a SUD but were not currently in treatment. These participants were recruited by word of mouth, and diagnosis of SUD in parents was obtained via a structured interview of all DSM-IV axis I disorders (Composite International Diagnostic Interview; CIDI; Robins, et al., 1989), performed by a trained interviewer.

Clinical staff at Bouman GGZ informed patients with children in the targeted age range of the study and gave them an information brochure. If the patient consented to being contacted, one of the researchers telephoned the patient to explain the study, answer any questions, and confirm eligibility. If the patient and his/her child agreed to participate, an appointment was made for the test session. More than one child per family was allowed to participate in the study.

Written informed consent was obtained from all participants and their parents and participants received a gift certificate. The Erasmus University Medical Center Ethics Committee approved the study.

Psychosocial stress procedure

Participants in the general population sample completed the psychosocial stress procedure at the Erasmus University Medical Center in Rotterdam or at temporary testing locations nearer to their

homes. CPSUDs participated in an EEG study just before the psychosocial stress procedure, and both took place at the Erasmus University in Rotterdam.

Stress procedure sessions commenced with an explanation of the procedure by the experiment leader. After the completion of a questionnaire set, the electrodes of the electrocardiogram were attached and participants were told to breathe normally and to relax. After a ten minute pre-task rest period, the social stress tasks began, which were characterized by uncontrollability and social-evaluative threat, thus designed to elicit a stress reaction (Dickerson & Kemeny, 2004). These tasks entailed a mental arithmetic task (i.e. mental subtraction; 4 min), a public speaking task (imagine that one was accused of stealing from the school/workplace cafeteria, response in front of the experiment leader and a video camera; 8 min mental preparation, 6 min speech) and a computer mathematics task (mentally ordering numbers; 5 min; see Dieleman, van der Ende, Verhulst, & Huizink, 2010 for full details on the procedure). The session ended with a five minute recovery period and a relaxing nature documentary (25 min). Please see Figure 1.1 for a depiction of the procedure.

Heart rate, blood pressure, galvanic skin conductance and respiration were monitored constantly throughout the procedure. Of these, only heart rate and respiration data were used in the present thesis. Heart rate was averaged for each of the periods, and RSA was averaged for the last three minutes of each of the periods when speaking did not occur (i.e. all resting periods, the preparation part of the public speaking task and the computer mathematics task). Salivary cortisol was collected after the pre-task rest period, each of the tasks, and during the middle and end of the documentary. Perceived stress was self-reported after the pre-task rest, each of the tasks and at the end of the procedure.

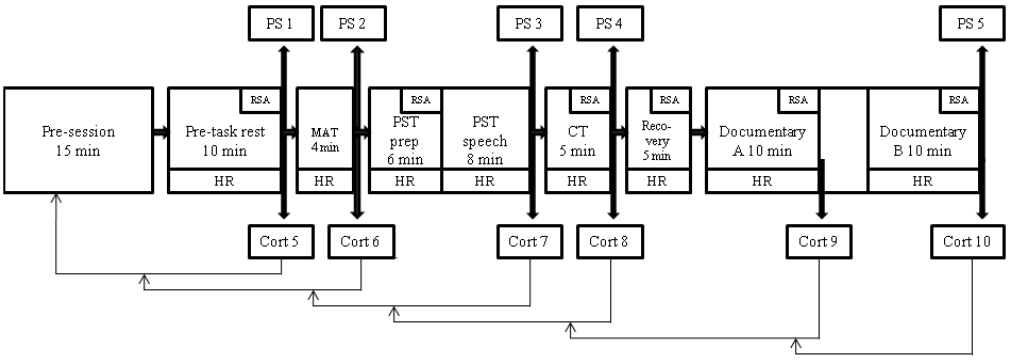


Figure 1.1. Schematic depiction of the stress procedure

Notes. PS = perceived stress; RSA = respiratory sinus arrhythmia; HR = heart rate; MAT = mental arithmetic task; PST = public speaking task; prep = preparation; CT = computer task; Cort 5-Cort 10 = cortisol tubes 5 through 10. Arrows extending from Cort 5- 10 point to moments during the procedure to which cortisol levels correspond, due to the delay in observable cortisol increase after the onset of the stressor (Sapolsky, et al., 2000).