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

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Chapter 5

Physiological stress responses as a vulnerability factor for externalizing symptoms and substance use in children of parents with a substance use disorder

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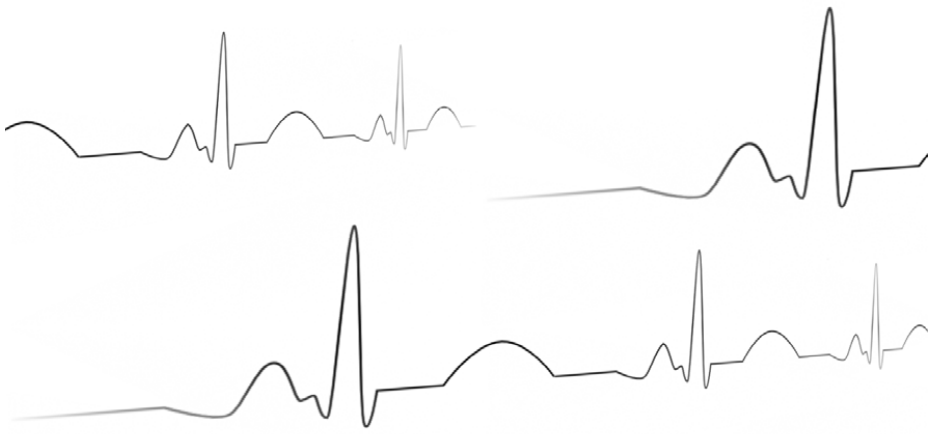

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Chapter 5

ABSTRACT

Background

Children of parents with a substance use disorder (CPSUD) are at increased risk for developing various forms of psychopathology, especially externalizing symptoms and risky substance use. These children may manifest markers that indicate a vulnerability to psychopathology. Better understanding of such vulnerability markers will help illuminate the developmental pathway between familial risk and psychopathology. This study examined autonomic nervous system (ANS) activity as a potential vulnerability marker, or endophenotype, in CPSUDs and a control sample.

Method

Respiratory sinus arrhythmia (RSA) and heart rate (HR) were measured continuously during a psychosocial stress procedure. Participants were adolescents between 11 and 20 years, of whom 81 were CPSUDs and 524 were controls. Resting RSA and HR, and RSA and HR reactivity and recovery were examined in relation to familial risk status, externalizing symptoms, and tobacco, alcohol and cannabis use.

Results

Multiple outcome regression analyses showed that externalizing symptoms were independently predicted by positive familial risk status (p<.001), and blunted HR reactivity (p<.001) and recovery (p<.001). Cannabis use was predicted by positive familial risk status only (p=.008). Tobacco use was independently predicted by blunted HR reactivity (p=.006) and recovery (p<.001). Furthermore, the relation between familial risk status and externalizing symptoms (p<.001) and tobacco use (p=.004) was mediated by HR recovery. Alcohol use was not related to familial risk status or ANS activity.

Conclusion

Our findings indicate evidence for blunted heart rate recovery as an endophenotype of tobacco use and externalizing symptoms.

INTRODUCTION

Since the beginning of the previous century, scientists have affirmed the severity of the ramifications of having a parent with a substance use disorder (SUD; Keynes, 1911). Children of parents with a SUD (CPSUD) are at increased risk for developing psychopathology (Cloninger, 1987), the most prevalent and widely reported forms being externalizing symptoms and risky substance use (Chassin, et al., 1991; Johnson & Leff, 1999; Stanger, et al., 1999; Tessner & Hill, 2010).

Although CPSUDs clearly show a higher prevalence of psychopathology, the developmental pathway between being the child of a parent with a SUD and manifesting externalizing symptoms and risky substance use remains to be elucidated. Endophenotypes, or vulnerability markers, may aid in the clarification of the development of psychopathology in CPSUDs. One potential endophenotype is the physiological stress system, which can be indexed by the autonomic nervous system (ANS).

The ANS consists of the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The vagus, a primary component of the PNS, maintains homeostasis and supports social engagement during rest, thereby keeping heart rate (HR) low (Porges, 1995; Porges, 2007). When an organism is confronted with a stressor, the most immediate response involves vagal withdrawal, which increases HR, indicating the organism's preparedness to respond to an anticipated stressor. If this response is insufficient, the SNS is activated, entailing the fight-or-flight response, elevating HR (further). Respiratory sinus arrhythmia (RSA) is a valid index of vagal tone (Porges, 1995).

Optimal responding entails rapid, pronounced vagal withdrawal, a rapid and pronounced increase in HR, and following the stressor, a rapid and pronounced decrease in HR and a return to higher vagal levels (Porges, 2007; Turner, 1994). Low resting vagal tone and reduced withdrawal are thought to signify emotion dysregulation (El-Sheikh & Erath, 2011; Porges, 2007). Low resting HR and reduced HR reactivity are thought to indicate fearlessness, and thereby reduced inhibition to engaging in delinquent or antisocial behavior (Raine, 1993). Another theory associates low physiological reactivity with a heightened tendency to engage in sensation seeking behaviors, due to an inherent hypo-arousal which leads individuals to actively seek out stimulation in order to achieve a state of normalized physiological arousal (Goeders, 2003; Majewska, 2002).

The physiological stress response seems to be a viable endophenotype because previous work has shown it to be divergent in individuals with SUDs (Back, Brady, Jackson, Salstrom, & Zinzow, 2005; Panknin, et al., 2002; Sinha, et al., 2009) and externalizing disorders (Raine, 1993). However, whether aberrations in the physiological stress response are underlying biological characteristics of the patients or whether they are the consequence of chronic and heavy substance use remains unresolved.

Some research supports the idea of underlying biological characteristics, as early studies indicated abnormal physiological stress responding in CPSUDs (Finn & Pihl, 1987; Moss, et al., 1995). However, studies investigating specifically HR and RSA reactivity have been equivocal. Children of parents who indicated problem drinking did not differ from controls pertaining to RSA (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). HR *hypo*-reactivity to psychosocial stress has been reported in adult CPSUDs (Sorocco, et al., 2006). In other studies investigating HR responses to unavoidable shock in adult CPSUDs, *hyper*-reactivity was generally found (Pihl, et al., 1990), and this response pattern was confirmed in a sample of adolescent CPSUDs (Harden & Pihl, 1995). These studies varied considerably with regard to the age of participants, the type of stressor used and the sample size. Moreover, very few studies have investigated RSA responses in CPSUDs. Therefore, consensus is lacking at this point as to the nature of ANS responses to stress in CPSUDs.

The aim of the present study was to examine whether ANS activity could be a potential vulnerability marker on the developmental pathway between being the child of a parent with a SUD and externalizing and substance use behavior during adolescence. We first examined an *additive model*, investigating to what extent familial risk status and ANS activity were independently predictive of externalizing symptoms and substance use. We hypothesized being a CPSUD and portraying low resting ANS activity and reduced ANS responding would predict externalizing symptoms and substance use, based on findings from previous research (i.e. Chassin, et al., 1991; El-Sheikh & Erath, 2011; Fairchild, et al., 2008; Harden & Pihl, 1995; Hastings, et al., 2007; Ortiz & Raine, 2004; Popma, et al., 2006; Van Goozen, et al., 2000).

From earlier studies, it seems that higher resting vagal tone and more pronounced vagal withdrawal buffer children from maladaptive outcomes in situations of adverse familial

environments (Diamond, et al., 2012; El-Sheikh & Erath, 2011; El-Sheikh, Hinnant, & Erath, 2011), however, only one study to our knowledge examined parental substance use as an index of adverse familial environment (El-Sheikh, 2001, 2005). Therefore, the second model that we examined was a *moderation model*, implicating an interaction term between familial risk status and resting ANS and ANS responding. We hypothesized that individuals who were CPSUDs and who manifested low resting ANS and blunted ANS responding would portray the highest number of externalizing symptoms and be most likely to report substance use.

Thirdly, we investigated a *mediation model* (MacKinnon & Luecken, 2008). Research has shown that prolonged exposure to stress may be related to subsequent physiological hypo-arousal (Burgess, Marshall, Rubin, & Fox, 2003). Being the child of a parent with a SUD can be a severe stressor that may last many years, therefore it is plausible that CPSUDs, as a consequence of experiencing stress over a lengthy period of time, develop physiological hypo-arousal. Alternatively, hypo-arousal could be the consequence of genetic effects.

METHODS

Participants

Children of parents with a substance use disorder

The sample of CPSUDs consisted of 81 adolescents (11-20 years old) 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 tobacco; APA, 2000). The majority of these adolescents were recruited from outpatient Bouman GGZ clinics, where their parents were in treatment for SUD(s). SUD diagnoses in parents were obtained from the clinical staff at Bouman GGZ. 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. Some participants (N=6) had parents who were diagnosed with 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 1 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 by a researcher, one of the researchers telephoned the patient to explain the study, answer any questions, and confirm eligibility. If the patient and adolescent agreed to participate, an appointment was made for the testing session. More than one child per family was allowed to participate in the study.

Controls

Controls were part of a larger sample of children and adolescents that participated in a longitudinal Dutch general population study (Tick, et al., 2007). For this larger study, participants were randomly drawn from registers of 35 representative municipalities in the province of South Holland including urban and rural areas. As part of the second wave of measurements, 711 participants (aged 7-20 years) participated in a psychosocial stress procedure. Of these, data on resting, reactivity and/or recovery measures of HR and/or RSA were available for 636 individuals. In order to obtain groups with the same range of ages, we excluded all control subjects younger than 11 years old, which led to a sample of n=532. Finally, we excluded those whose parents had been diagnosed with a SUD (n=8), leading to a control sample of 524 subjects. For a detailed description of the study sample and design, please see Huizink, et al. (2012).

Procedure

The psychosocial stress procedure commenced with an explanation of the procedure by the researcher. After the completion of two questionnaires, 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 (first period), the social stress tasks began, which were designed to elicit a stress reaction (Dieleman, et al., 2010; Evans, et al., 2012; Huizink, et al., 2012). These tasks entailed a mental arithmetic task, a public speaking task (preparation and speech) and a computer mathematics task (second, third, fourth and fifth periods). The session ended with a five minute resting period (sixth period) and a relaxing nature documentary (25 minutes in two ten minute blocks; seventh and eighth periods). Figure 5.1 depicts the procedure schematically. Written informed consent was obtained from all participating adolescents and their parents, and adolescents received a gift certificate. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Center.

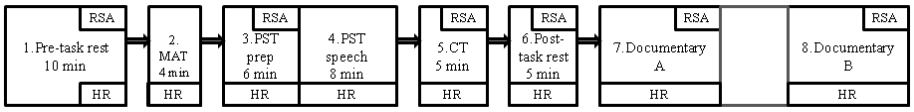

Figure 5.1. Stress procedure during which heart rate was measured continuously

Note. Blue boxes show when RSA was measured (3 min blocks), green boxes show when HR was measured. RSA=respiratory sinus arrhythmia; HR=heart rate; MAT=mental arithmetic task; PST=public speaking task; prep=preparation; CT= computer task

Measures

Externalizing symptoms

The Dutch version of the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) was completed by the mothers of the participants. For many participants, fathers also completed the CBCL, and adolescents completed the Dutch version of the Youth Self Report (YSR; Achenbach & Rescorla, 2001). In order to determine the number of externalizing symptoms, scores on three subscales (conduct, oppositional defiant and attention deficit hyperactive symptoms) were summed. Number of internalizing symptoms was similarly achieved (using subscales affective, anxiety and somatic disorders). Father and adolescent reports, and mother-reported internalizing symptoms were used only in the multiple imputation procedure (see statistical analysis) and were not examined as variables of interest.

Substance use

We assessed substance use with a Substance Use Questionnaire, completed by the adolescent. Ever use of *alcohol* (at least one glass) and *cannabis* were coded as yes (x=1) or no (x=0). *Tobacco use* was coded dichotomously as those who have never smoked, have smoked one or two cigarettes ever, currently smoke once in a while, or had quit (x=0); and those who smoke every day (x=1).

Heart rate

HR was measured using a three-lead electrocardiogram (ECG), monitored constantly throughout the entire stress procedure. The ECG was sampled at 512 Hz and stored on a flashcard by means of a portable digital recorder (Vitaport^{TM} System; TEMEC Instruments B.V., Kerkrade, The Netherlands). After completion of the recording, all physiological data were imported and processed on a Personal Computer using a Vitascore^{TM} software module (TEMEC Instruments BV, Kerkrade, The Netherlands). A customized software program calculated the interbeat intervals (IBI) of the ECG using R-top detection, resulting in IBI time series. This time series was inspected for detection and removal of artifacts. HR time series were calculated from these IBI time series and expressed in beats per minute (bpm); the HR time series were subsequently averaged per period during the stress procedure. To determine *resting HR*, we used the last period of the entire procedure (eighth period) because on average, HR was lowest during this period, and therefore is presumed to indicate a resting level most accurately. In accordance with previous studies (i.e. Willemen, et al., 2009), *HR reactivity* was calculated by subtracting the last three minutes of pre-task HR from the maximum HR during any of the three stress tasks. *HR recovery* pertained to the difference between the maximum HR during any of the three stress tasks and HR during the first post-task rest period (sixth period).

Respiratory sinus arrhythmia

In order to compute an index of RSA, the HR time series during the pre-task rest period, the post-task recovery period, the preparation part of the public speaking task and the computer task (i.e., those periods when speaking did not occur) were scrutinized for stationarity. The HR time series were subsequently subjected to a discrete Fourier transformation, based on non-equidistant sampling of the R-wave incidences (CARSPAN program, Groningen, The Netherlands; Mulder, et al., 1988; Van Steenis, et al., 1994), to yield power spectra of the rhythmic oscillations over a frequency range of 0.02-0.50 Hz, with a resolution of 0.01 Hz. For each period, the power in the high frequency band (0.14-0.5 Hz) of the HR time series was calculated as an index of RSA. The data were log transformed in order to obtain normal distributions. Similar to HR, RSA during the last period of the procedure (eighth period) was used to indicate *resting RSA*. *RSA reactivity* was calculated by subtracting the minimum RSA during either of the two stress tasks from pre-task RSA. For *RSA recovery*, we subtracted the minimum during the stress tasks from post-task RSA (sixth period).

Covariates

In previous studies examining physiological stress reactivity, age (Phillips, et al., 2009), sex (Hardie, et al., 2002), ethnicity (Musante, et al., 2000), body mass index (BMI; Carroll, et al., 2008), SES (Sorocco, et al., 2006), and urbanicity (Armstead, et al., 2010), have been included as covariates. SES was based on the higher occupational level of either parent (Statistics, 2010) and coded into low (x=0), average (x=1) or high (x=2). Age, sex (boy: x=0; girl: x=1) and ethnicity (of Dutch origin, x=0; of non-Dutch origin, x=1) were assessed using a demographics self-report questionnaire. Height and weight were measured prior to the test session and used to calculate BMI. Urbanicity was based on the population rate of the home city/town of the participant at the time of the test session, coded as rural (x=0), town (>10,000 inhabitants; x=1) or urban (>100,000 inhabitants; x=2; Lederbogen, et al., 2011). Population statistics were based on online national archives (Statistics, 2011).

Statistical analysis

To confirm that the stressful tasks induced an increase in HR and a decrease in RSA compared to the pre-task rest period, we performed a manipulation check by way of two repeated measures analyses of variance (RM-ANOVA) in the entire sample, one for HR (1x6) and one for RSA (1x4), in IBM SPSS statistics version 20.

In the main analysis, we used a multiple imputation procedure to handle missing data on all outcome variables and covariates. All continuous variables were centered, and categorical variables were set to x=0 as the first category. As the main analysis, we examined possible interrelations between familial risk status, ANS activity and externalizing symptoms and substance use by specifying three types of models in Mplus version 6.11 (Muthén & Muthén, 1998-2011). In each model, externalizing symptoms, and tobacco, alcohol and cannabis use were outcome variables and familial risk status and ANS activity were independent variables. We specified each model for resting RSA, RSA reactivity, RSA recovery, resting HR, HR reactivity and HR recovery separately. Because siblings were included in the CPSUD group, and heritability of HR reactivity has been shown (Mueller, et al., 2012), we used the CLUSTER option to account for familial dependency. All paths were controlled for age, sex, ethnicity, BMI, SES and urbanicity.

We first formulated an *additive model* by examining the extent to which familial risk status and ANS activity predicted externalizing symptoms and substance use. Next, we

examined a *moderation model* by adding an interaction term of familial risk status by ANS activity variables to the additive model. Lastly, we examined a *mediation model*. In addition to the paths defined in the additive model, we examined the direct path between familial risk status and the ANS activity variables. If the direct paths between familial risk status and ANS activity, and ANS activity and outcome were significant, we also examined the indirect path of familial risk status via ANS activity to externalizing symptoms and substance use. Because we ran several models, we considered results to be significant at $p<.01$ in order to minimize the chance of type I errors. If more than one model was significant for the same combination of predictors and outcome variables, we assessed which model fit the data better using the Akaike Information Criterion (AIC).

RESULTS

First, descriptives of and correlations between variables were calculated (see Tables 5.1 and 5.2, respectively). T tests for independent means and χ^2 tests were used to examine differences between CPSUDs and controls.

Manipulation check

All participants taken together, RM-ANOVAs showed that HR increased during the tasks relative to the pre-task rest period (see Table 5.3). RSA was also increased as opposed to the expected RSA withdrawal. This could be due to anticipation effects, which may be particularly strong in RSA (see also (Oldehinkel, et al., 2011), so we examined the tasks relative to the post-task rest period. These contrasts showed that RSA was decreased, relative to the sixth period, during the computer task.

Table 5.1. Descriptive statistics for each familial risk status group

	Control		**CPSUD**		**Group differences**
	N	Mean (SD) or frequency (%)	N	Mean (SD) or frequency (%)	χ^2 or *t*
HR pre-task	429	77.49(10.00)	81	75.16(8.89)	1.96
HR mental arithmetic task	439	82.70(11.70)	80	78.28(8.28)	3.23**
HR public speaking preparation	437	79.31(10.88)	81	76.86(8.98)	1.91
HR public speaking task	436	84.23(12.03)	81	81.28(9.67)	2.08*
HR computer task	434	78.82(10.98)	79	74.65(8.54)	3.21**
HR post-task rest	434	74.11(9.50)	80	74.00(8.90)	0.09
HR documentary A (first 10 min)	432	73.20(9.64)	80	72.60(8.44)	0.53
HR documentary B (last 10 min; resting HR)	432	72.19(9.43)	79	71.44(8.29)	0.67
RSA pre-task	378	7.51(0.93)	78	7.76(0.82)	-2.18*
RSA public speaking preparation	379	7.71(0.87)	79	7.88(0.85)	-1.60
RSA computer task	378	7.61(0.94)	77	7.95(0.86)	-2.97**
RSA post-task rest	373	7.75(0.95)	77	7.97(0.88)	-1.92
RSA documentary A (first 10 min)	375	7.67(0.94)	78	8.03(0.78)	-3.15**
RSA documentary B (last 10 min; Resting RSA)	359	7.73(0.94)	73	8.08(0.82)	-2.91**
HR max stress	439	86.31(12.16)	81	82.39(9.54)	2.75**
RSA max stress	386	7.43(0.89)	79	7.74(0.84)	-2.82**
HR reactivity	429	8.81(8.69)	81	7.23(7.05)	1.54
HR recovery	434	12.25(7.93)	80	8.40(7.03)	4.05***
RSA reactivity	376	0.08 (0.64)	78	0.03 (0.48)	0.75
RSA recovery	372	0.32 (0.59)	77	0.23 (0.47)	1.33
Externalizing symptoms	386	0.84 (0.72)	65	1.46 (1.07)	-5.92***
Tobacco use (Never smoked, 1 or 2 ever, quit/Smokes daily)	414	90.8/9.2	78	76.9/23.1	12.57***

Table 5.1 continued.

	Control		**CPSUD**		**Group differences**
	N	Mean (SD) or frequency (%)	N	Mean (SD) or frequency (%)	χ^2 or *t*
Alcohol use (Never use/Ever use)	441	34.7/65.3	78	26.9/73.1	1.80
Cannabis use (Never use/Ever use)	412	83.5/16.5	77	67.5/32.5	10.73**
Age	443	15.52 (2.68)	81	16.09 (2.48)	-1.78
Sex (Boys/Girls)	443	44.5/55.5	81	50.6/49.4	1.04
Ethnicity (Dutch/Non-Dutch origin)	443	82.4/17.6	80	91.3/8.8	3.91*
Body mass index	434	21.19 (3.60)	74	20.50 (3.41)	1.54
SES (Low/Average/High)	441	4.8/51.0/44.2	72	2.8/73.6/23.6	12.73**
Urbanicity (Rural/Town/Urban)	443	13.1/57.3/29.6	81	14.8/55.6/29.6	0.19

*p<.05, **p<.01, ***p<.001; HR=heart rate; RSA=respiratory sinus arrhythmia; SES = socioeconomic status

Table 5.2. Correlations between all independent and dependent variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.HR resting																
2.HR reactivity	.05															
3.HR recovery	.02	**.80**														
4.RSA resting	**.26**	-.02	-.02													
5.RSA reactivity	.03	**.19**	.07	.04												
6.RSA recovery	.02	.02	**.15**	**.20**	**.49**											
7. Externalizing	-.03	**-.21**	**-.28**	.06	.02	-.02										
8. Tobacco use	**-.13**	*-.11*	**-.13**	*.10*	-.06	-.05	**.19**									
9. Alcohol use	**-.24**	.08	*.09*	*.10*	*-.10*	-.05	-.06	**.25**								
10. Cannabis use	**-.15**	-.01	.01	.06	-.06	-.00	.06	**.54**	**.34**							
11. Fam. risk status	-.02	-.07	**-.17**	**.16**	-.04	-.06	**.22**	**.16**	.06	**.15**						
12. Age	**-.17**	.06	.07	.03	-.08	**-.13**	*-.11*	**.30**	**.64**	**.40**	.07					
13. Sex	*.09*	.06	**.15**	.06	.03	**.23**	*-.10*	.08	.06	-.01	-.05	.05				
14. Ethnicity	*.09*	-.05	-.08	-.07	-.00	-.02	.06	-.01	*-.09*	-.06	*-.09*	.05	-.01			
15. BMI	-.04	*-.10*	-.07	*.11*	-.04	.02	-.06	**.15**	**.27**	**.15**	-.06	**.34**	**.17**	.07		
16. SES	-.02	**.15**	**.22**	.00	.04	.03	**-.13**	*-.12*	.06	-.02	**-.13**	.01	.05	*-.11*	**-.12**	
17. Urbanicity	-.00	**-.12**	*-.11*	-.05	-.05	-.09	.01	.03	-.02	-.04	-.01	.06	-.00	**.33**	.05	.06

$p<.01$: **bold** $p<.05$: *italics*; HR=heart rate; RSA=respiratory sinus arrhythmia; Fam.=familial; BMI=body mass index; SES=socioeconomic status

Additive models

Familial risk status significantly predicted number of externalizing symptoms and cannabis use, but not tobacco or alcohol use. CPSUDs exhibited more externalizing symptoms, and were more likely to have used cannabis. The relation between positive familial risk status and a higher likelihood of using tobacco daily was marginally significant (p=.01). HR reactivity and recovery were significantly related to number of externalizing symptoms and tobacco use. Adolescents who exhibited blunted HR reactivity and recovery showed more externalizing symptoms and were more likely to smoke daily. None of the RSA variables were related to any of the outcome variables. Table 5.4 presents an overview of each of the models.

Moderation models

None of the interaction terms reached statistical significance. Thus, the relation between familial risk status and externalizing symptoms and substance use did not depend on ANS activity.

Mediation model

Because familial risk status was not related to the potential mediators resting HR, RSA and HR reactivity, and RSA recovery, and significance of this pathway is a precondition for testing the indirect pathway, these mediation models were not tested. Familial risk status was related to resting RSA, but resting RSA was not related to any outcomes, therefore this mediation model was also not tested. Furthermore, the direct paths between HR recovery and alcohol and cannabis use were not significant, therefore we only examined the potential mediator HR recovery with the outcome variables externalizing symptoms and tobacco use. Familial risk status was a significant predictor of HR, such that CPSUDs showed blunted HR recovery following the stressful tasks. The indirect path of familial risk status via HR recovery to externalizing symptoms was significant (p<.001), as was the indirect path from familial risk status via HR recovery to tobacco use (p=.004). HR recovery thus mediated the relation between familial risk status and externalizing symptoms and tobacco use.

For the interrelations between familial risk status, HR recovery and externalizing symptoms, both an additive and a mediation model explained the data. We then examined the AIC in order to compare the models. The AIC for the additive model was 1042.15, and for the

mediation model 4176.65. This suggests that the additive model may have been a better fit of the data.

Table 5.3. Statistics for manipulation check: RM-ANOVAs of physiological stress levels across the psychosocial stress procedure

	Model/contrast statistics		**Direction of effect**
	F	*p*	
Heart rate			
WS main effect	329.35	<.001	
Contrasts: relative to first period			
MAT	298.45	<.001	increase
PST preparation	111.75	<.001	increase
PST speech	412.19	<.001	increase
Computer task	46.98	<.001	increase
Respiratory sinus arrhythmia			
WS main effect	23.04	<.001	
Contrasts: relative to first period			
PST preparation	47.80	<.001	increase
Computer task	8.15	<.01	increase
Contrasts: relative to sixth period			
PST preparation	1.94	.164	decrease
Computer task	16.43	<.001	decrease

Note: Model statistics are Greenhouse-Geisser statistics to correct departures from sphericity. Samples sizes are *n*=507 (heart rate) and *n*=430 (RSA). WS = within subjects; MAT = mental arithmetic task; PST = public speaking task

Table 5.4. Results of linear regression analyses, all models predict number of externalizing symptoms

	Additive models							
Outcomes	**Externalizing**		**Alcohol use**		**Cannabis use**		**Tobacco use**	
Predictor	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)	*p*
Familial risk status	**0.63(0.13)**	**<.001**	0.19 (0.39)	**.**623	**1.03 (0.39)**	**.008**	1.04 (0.41)	.010
Resting HR	-0.00 (0.01)	.584	-0.03 (0.02)	.031	-0.03 (0.02)	.053	-0.03 (0.02)	.103
HR reactivity	**-0.02 (0.01)**	**<.001**	0.02 (0.02)	.322	-0.01 (0.02)	.507	**-0.05 (0.02)**	**.006**
HR recovery	**-0.03 (0.01)**	**<.001**	0.02 (0.02)	.392	-0.01 (0.02)	.553	**-0.09 (0.03)**	**<.001**
Resting RSA	0.05 (0.05)	.247	0.25 (0.18)	.164	0.29 (0.16)	.069	0.34 (0.20)	.083
RSA reactivity	-0.03 (0.07)	.703	-0.37 (0.28)	.198	-0.05 (0.24)	.846	-0.26 (0.27)	.335
RSA recovery	-0.05 (0.08)	.559	0.21 (0.29)	.465	0.31 (0.26)	.235	-0.12 (0.29)	.685
	Moderation models							
Outcomes	**Externalizing**		**Alcohol use**		**Cannabis use**		**Tobacco use**	
Predictor	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)	*p*
FRS by Resting HR	0.02 (0.02)	.167	-0.03 (0.04)	.452	-0.03 (0.04)	.507	0.03 (0.05)	.499
FRS by HR reactivity	-0.01 (0.02)	.619	0.08 (0.05)	.110	0.03 (0.05)	.558	0.04 (0.05)	.408
FRS by HR recovery	0.00 (0.02)	.841	0.07 (0.05)	.140	0.04 (0.05)	.411	0.01 (0.05)	.857
FRS by Resting RSA	-0.17 (0.15)	.274	0.44 (0.47)	.347	-0.48 (0.43)	.257	-1.06 (0.42)	.012

FRS by RSA reactivity	0.20 (0.22)	.376	0.85 (0.69)	.219	0.40 (0.77)	.601	0.64 (0.79)	.418
FRS by RSA recovery	-0.06 (0.26)	.828	0.54 (0.80)	.498	-0.17 (0.80)	.832	-0.77 (0.80)	.335
	Mediation models							
Outcomes	**Resting HR**		**HR reactivity**		**HR recovery**			
Predictor	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)	*p*		
Familial risk status	-0.15 (1.17)	.897	-0.07 (0.05)	.112	**-3.88 (0.90)**	**<.001**		
Outcomes	**Rest RSA**		**RSA reactivity**		**RSA recovery**			
Predictor	Estimate(SD)	*p*	Estimate(SD)	*p*	Estimate(SD)			
Familial risk status	**0.41 (0.12)**	**.001**	-0.01 (0.07)	.928	-0.05 (0.06)	.468		
Outcomes	**Externalizing**		**Tobacco use**					
Predictor	Estimate(SD)	*p*	Estimate(SD)	*p*				
Indirect pathway	**0.09 (0.03)**	**.001**	**0.32 (0.11)**	**.004**				

p<.002: **bold**; HR=heart rate; RSA=respiratory sinus arrhythmia; FRS=familial risk status

Note: sample sizes vary by ANS activity variable (Resting HR: *n*=444; HR reactivity: *n*=447; HR recovery: *n*=447; Resting RSA: *n*=387; RSA reactivity: *n*=406; RSA recovery: *n*=403); values for the main effects in mediation and moderation models were similar to those reported in the additive model.

Chapter 5

DISCUSSION

In a sample of CPSUDs and matched controls, we examined RSA and HR across a psychosocial stress procedure. We utilized additive, moderation and mediation models to explore the interrelations between familial risk status, ANS activity, and externalizing symptoms and substance use. Additive models signify independent effects of the predictor on outcome variables. A moderation effect implies that the relation between familial risk status and outcome variables depends on physiological stress responses, while mediation implies causality, thus aberrant physiological responses could explain the relation between familial risk status and the outcomes. Familial risk status best explained cannabis use in adolescents; CPSUDs were more likely to have used cannabis. Tobacco use was best explained by single predictors and a mediation model. Both blunted HR reactivity and recovery were related to a higher likelihood of using tobacco daily. Furthermore, though CPSUDs were only marginally more likely to use tobacco daily, blunted HR recovery mediated this relation, thus, blunted HR recovery partially explained the mechanism by which CPSUDs were more likely to use tobacco daily. Externalizing symptoms were explained by additive models and a mediation model. Familial risk status, HR reactivity and HR recovery were independently related to externalizing symptoms; CPSUDs showed more externalizing symptoms, just as adolescents with blunted HR reactivity and recovery. There was also some evidence that HR recovery mediated the relation between familial risk status and externalizing symptoms, however, the AIC of the additive model was lower, suggesting a better fit to the data.

Consistent with earlier studies, CPSUDs in our sample reported more externalizing symptoms and were more likely to use cannabis (e.g. Chassin, et al., 1991; Johnson & Leff, 1999; Stanger, et al., 1999; Tessner & Hill, 2010). The relation between familial risk status and tobacco use was only marginally significant, though in the expected direction of CPSUDs being more likely to use tobacco daily. Also in line with previous research, blunted HR reactivity and recovery were related to more externalizing symptoms and an increased likelihood of smoking daily (Evans, et al., 2012; Fairchild, et al., 2008; Hastings, et al., 2007; Ortiz & Raine, 2004; Phillips, et al., 2009; Popma, et al., 2006; Van Goozen, et al., 2000). Intriguingly, we found evidence for significant mediation effects of HR *recovery*, but not HR *reactivity*. Previous research generally found blunted HR recovery to indicate health risks (e.g. Cole, et al., 1999; Georgoulias, et al., 2003). A reasonable interpretation of the present findings may then be that, blunted HR recovery, like blunted HR reactivity, is indicative of a

less optimally functioning stress response system. The ANS may be less able to recover from exposure to stress, thereby maintaining a higher level of arousal for longer than is necessary or healthy. This is consistent with suggestions that physiological stress response dysregulation in adolescents may signal vulnerability to psychopathology (Stroud, et al., 2009).

Despite a relatively large body of literature on RSA activity relating to psychopathology, and moderating the relation between adverse familial environments and child/adolescent psychopathology (e.g. Beauchaine, 2001; El-Sheikh & Erath, 2011; Porges, 2007), in the present study, RSA activity was not related to any of the outcome measures. It is not entirely clear why these relations were not evident in our sample. In all likelihood, this had to do with the stressful tasks during which RSA was measured not being sufficiently stressful to elicit a RSA response. It is not reliable to measure the high frequency band of power spectral analysis (our index of RSA) during speech, therefore, the analyses on RSA responsivity were limited to the preparation part of the public speaking task and the computer task. It seems logical, and is confirmed with HR data, that the preparation part of the public speaking task and the computer task were less stress-evoking than the mental arithmetic task and speech part of the public speaking task.

Interestingly, RSA increased in response to the stressful tasks, as did HR. Because the heart is innervated by both branches of the ANS, an increase in HR suggests SNS activation and/or PNS withdrawal, and often, PNS withdrawal coincides with SNS activation, although this is not always the case (Berntson, et al., 1991). Simultaneous PNS and SNS activation, or coactivation (Berntson, et al., 1991), as was the case in our sample, has previously been shown to be related to problem behavior and externalizing symptoms (Boyce & Ellis, 2005; El-Sheikh, et al., 2009; Hinnant & El-Sheikh, 2009), and may indicate incompetence in engaging in environmental demands (El-Sheikh & Erath, 2011). However, another explanation is that RSA was on average already low during the first period due to anticipation of the upcoming stressor, and therefore did not decrease further in response to the tasks. Indeed, the PNS responds immediately to an upcoming stressor (Porges, 2007). It is possible that participants were mentally preparing for the task already during the pre-task rest, which induced RSA withdrawal.

In comparison to the post-task rest period, RSA levels were significantly lower during the computer task, thus a recovery measure was on average in the expected direction. Although little research has examined RSA recovery, we had expected similar results as have

been reported with RSA reactivity, namely blunted RSA withdrawal being related to externalizing symptoms (El-Sheikh & Erath, 2011). Also, low resting RSA is often seen as an indicator of emotion dysregulation and to therefore be related to externalizing symptoms (Beauchaine, 2001; Porges, 2007).

This study must be considered in light of the following. Recovery was calculated as the difference between physiological activity during the stressful tasks and first post-task rest period. Difference scores provide more information than a single data point. However, calculated in this way, it may be difficult to differentiate between reactivity and recovery (Uchino, Smith, Holt-Lunstad, Campo, & Reblin, 2007). Often, recovery is calculated as the time to return to baseline (pre-task) levels, but in our design this was not possible. Secondly, these findings are cross-sectional in nature, and therefore preliminary. Further longitudinal research will be necessary in order to confirm these results.

In conclusion, HR recovery may be a potential endophenotype for tobacco use and possibly for externalizing symptoms. HR recovery from stress may be one of the mechanisms underlying the frequently observed finding that CPSUDs exhibit more externalizing symptoms and are more likely to smoke daily.

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