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

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Chapter 4

Alcohol and tobacco use and heart rate reactivity to a psychosocial stressor in an adolescent population

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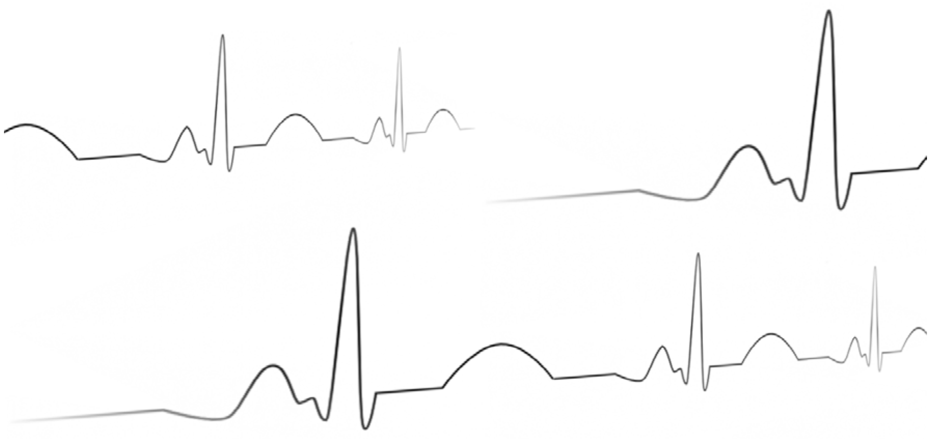

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ABSTRACT

Background

Few studies have investigated physiological stress (re)activity in relation to substance use, especially in adolescents. Using substances is one way to stimulate physiological arousal, therefore inherent hypo-arousal may be associated with substance use in adolescents. The purpose of this study was to examine the relation of autonomic nervous system (ANS) activity with alcohol and tobacco use in adolescents.

Method

ANS activity and perceived stress during a social stress procedure were examined in relation to substance use. Two hundred seventy-five Dutch adolescents from a general population study provided complete data. Heart rate was recorded continuously during a pre-task rest period, a stressful task period and a post-task recovery period. Alcohol and tobacco use were self-reported.

Results

Adolescents who consumed a medium and high number of alcoholic drinks per week (more than two) exhibited lower heart rates during the entire stress procedure as compared to those who consumed a low number of alcoholic drinks. Adolescents who smoked every day portrayed blunted heart rate reactivity to stress as compared to adolescents who smoked less frequently or not at all. Perceived stress was not related to alcohol or tobacco use.

Conclusion

Overall lower heart rate in adolescents who drank more and blunted heart rate reactivity to stress in those who used tobacco every day may indicate inherent hypo-arousal of the ANS system in those vulnerable to use substances more often. These adolescents may actively seek out substances in order to achieve a more normalized state of arousal.

INTRODUCTION

Adolescence is a critical developmental period regarding exploration behavior towards substances, with adolescents showing increased experimentation with alcohol and tobacco (Hardin & Ernst, 2009). Adolescents have generally not used substances in large amounts or for long periods of time, which facilitates the discernment of vulnerability markers for substance use problems. As it is less likely that the use of substances has caused lasting physical changes in this population, it is possible that differences found in those adolescents who are prone to use more alcohol and/or tobacco may be due to underlying, inherent factors.

Stress reactivity may be one potential vulnerability marker for the development of substance use disorders (SUDs). It has been related to SUDs in adults (for reviews see Goeders, 2003, Sinha, 2008). One view of this association describes the tendency of individuals to use substances in order to alleviate symptoms of stress, or hyper-arousal; self-medication hypothesis (Khantzian, 1985). 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 & 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); stimulus-seeking hypothesis (Huizink, et al., 2006; Zuckerman & Neeb, 1979). This hypo-arousal may be associated with multifarious stimulus-seeking behaviors, and has frequently been linked to externalizing behaviors (Raine, 2002), of which substance use may be one (Liu, et al., 2009).

Stress reactivity can be assessed by measuring the activation of the autonomic nervous system (ANS), which is responsible for the body's immediate response to stress and plays an important role in allostasis. In response to a stressor, the ANS prepares the body for action by increasing heart rate (HR), blood pressure and respiration. HR is a valid physiological index of stress (Porges, 1995) and in the field of SUD research, assessing HR reactivity to a psychosocial stressor is ideal due to its ecological validity.

In alcohol dependent patients, resting HR may be higher compared to social drinkers (Sinha, et al., 2009) and controls (e.g. Ingjaldsson, et al., 2003). In response to psychological stress, though, alcohol dependent patients could have lower HR reactivity (Panknin, et al., 2002). Pertaining to tobacco use, the acute effect of smoking entails an increase in HR

(Hasenfratz & Battig, 1992; James & Richardson, 1991; Pauli, Rau, Zhuang, Brody, & Birbaumer, 1993), though differences between habitual smokers and non-smokers in HR response to stress is unclear. Some studies reported no differences between smokers and non-smokers in resting HR (Kirschbaum, Strasburger, & Langkrär, 1993; Perkins, Grobe, Fonte, & Breus, 1992; Roy, et al., 1994) or in response to psychosocial stress (Back, et al., 2008; Childs & de Wit, 2009; Hughes & Higgins, 2010; Kirschbaum, et al., 1993; Perkins, et al., 1992; Tersman, Collins, & Eneroth, 1991). Others reported increased resting HR in smokers (al'Absi, et al., 2003; Phillips, et al., 2009; Sheffield, et al., 1997; Tsuda, et al., 1996) and attenuated HR responses to psychological stressors in large community samples of men (Sheffield, et al., 1997), in women (Girdler, et al., 1997; Straneva, et al., 2000) and with light as well as heavy smokers showing attenuated HR reactivity in comparison to non-smokers (Phillips, et al., 2009; Roy, et al., 1994).

As the above mentioned studies were performed in subjects who had already used substances heavily or were dependent on a substance, it is unclear whether the results point to underlying variation in the ANS, or whether substance use had affected this system directly. In order to minimize the possibility of heavy substance use dysregulating the ANS, it is important to perform studies in the general population, with subjects who have used substances relatively less. Studies that examined HR in response to stress in individuals with a family history (FH) of alcoholism, who had not yet developed problem drinking, led to differing results. One found that adults with a multigenerational FH of alcoholism, as compared to those with a unigenerational and negative FH, showed increased HR to unavoidable shock (Finn, Earleywine, & Pihl, 1992; Finn & Pihl, 1987). One study in boys found increased HR reactivity to a mental arithmetic task (Harden & Pihl, 1995). Contrastingly, another found blunted HR responses to psychosocial stress in adults with a FH of alcoholism as compared to those with a negative FH (Sorocco, et al., 2006). Clearly, more research is needed to elucidate this relationship.

Furthermore, as almost all of the above-mentioned studies were performed in adults, little is known about the relation between HR and substance use in adolescents. Substance use can be viewed as a manifestation of externalizing problems (symptoms of e.g. oppositional defiant and conduct disorder; Krueger, et al., 2002; Liu, et al., 2009). The relation between externalizing problems and HR has been well established; low resting HR is the best-replicated correlate of antisocial behavior in children and adolescents, and attenuated HR in response to a stressor is also well-confirmed (Ortiz & Raine, 2004). Thus, literature on the relation between externalizing problems and HR may provide insight into the relation that could be found between substance use during adolescence and HR reactivity, i.e. low resting HR and attenuated HR response to stress. Of interest here is whether HR is related to externalizing problems in general, or whether it is related specifically to substance use.

The goal of this study was to examine the relation between adolescent alcohol and tobacco use and HR (re)activity during a psychosocial stressor. We expected to find that adolescents who drank more alcohol and adolescents who used more tobacco would portray low resting HR and an attenuated HR response to the stressor. By entering number of externalizing problems into the model, we aimed to examine whether any found relation is specific for alcohol and tobacco use. Physiological responses are generally postulated to reflect subjective, or perceived stress (PS), responses (Thayer, 1970), however, convincing experimental evidence of this is limited (Oldehinkel, et al., 2011). Therefore, a second aim of this study was to examine whether HR and PS were related, and whether alcohol and tobacco use were related to PS. Based on findings in earlier studies in which HR did but PS responses did not vary by risk group (e.g. Fairchild, et al., 2008; Finn & Pihl, 1987), we hypothesized that HR would be related to PS, but that PS would not be related to alcohol and tobacco use in adolescents.

METHOD

Participants

The current sample of 275 14- to 20-year-old (M=17.22; SD=1.31) adolescents is part of a larger sample that participated in the South Holland 2 study, a large Dutch general population study of youth aged 6-20 years. For this larger study, children and adolescents were randomly drawn from registers of 35 representative municipalities in the Dutch province of South Holland including both urban and rural areas. At the second assessment wave, 536 individuals were eligible for the present study on the adolescent group, being between the ages of 14 and 20. Of these, 330 individuals took part in a stress procedure between January 2006 and March 2009. Of this sub-sample, complete HR data was available for 288 individuals (13% missing data, which is not irregular for research using HR data, see e.g. Dietrich, et al. (2007). This latter group did not differ from the 330 who participated in the stress procedure according to gender, SES or internalizing and externalizing symptoms, although participants with usable HR data were younger (p<.01). Complete HR as well as substance use data for the entire stress procedure was available for 275 adolescents. The latter group did not differ from the sample of 536 eligible individuals in terms of age, SES or internalizing symptoms, though female gender did significantly predict being included in the analysis ($p<.01$), and those included in the analysis reported fewer externalizing symptoms ($p<.05$). See Figure 4.1 for a flow chart of available data.

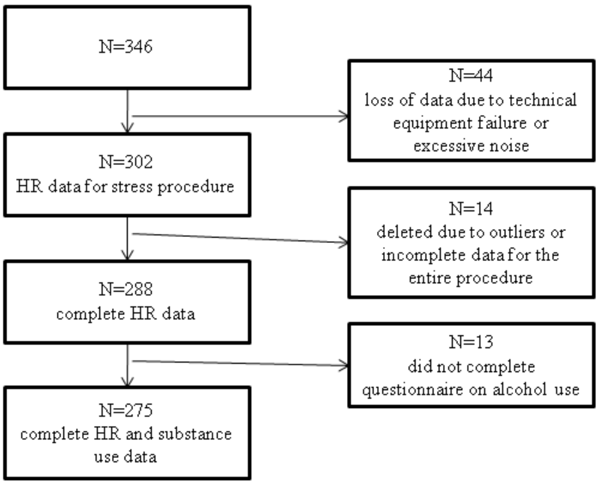

Figure 4.1. Flow chart of available data
Note. HR=heart rate

Procedure

Stress procedure sessions began at approximately 12 pm or 3 pm and commenced with an explanation of the procedure by the experiment leader. 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 rest period, the psychosocial stress tasks began, entailing mental arithmetic, public speaking and computer mathematics tasks (see Dieleman, et al., 2010 for full details on the procedure). The session ended with a five minute recovery period and a relaxing nature documentary (25 minutes). Figure 4.2 depicts the procedure schematically. Written informed consent was obtained from participating adolescents and their parents, and adolescents received a gift certificate. The study was approved by the Ethics Committee of the Erasmus University Medical Center.

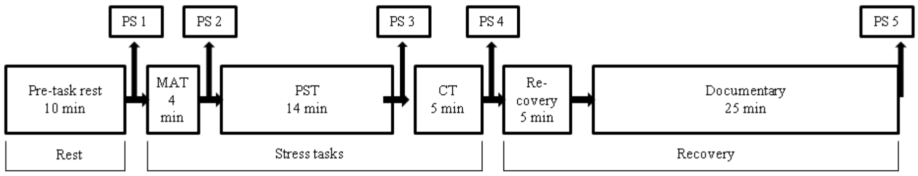

Figure 4.2. Stress procedure during which heart rate was measured continuously

Note. PS = perceived stress; MAT = mental arithmetic task; PST = public speaking task; CT = computer task

Measures

Alcohol and tobacco use

Self-reported alcohol use consisted of a composite of questions pertaining to the *number of days per week on which alcohol was usually drunk* multiplied by the *number of alcoholic drinks that was usually consumed per occasion*. This led to a continuous variable denoting the average number of alcoholic drinks consumed per week. Subjects were divided into three groups according to this (based on third percentiles; (Hillers & Massey, 1985; Murray, et al., 2002) which led to the variable group of Number of Drinks per Week (gNDW). Those who drank two alcoholic drinks per week or less (N=93) were considered Low Quantity (per week) Drinkers; between three and six (N=88) Medium Quantity Drinkers; and seven or more (N=69) High Quantity Drinkers. Table 4.1 describes additional alcohol use history variables.

Frequency of tobacco use was based on one multiple choice self-report question (*Have you ever smoked cigarettes?*). Three groups were formed based on the answer categories (group of Frequency of Tobacco Use; gFTU): Non-smokers (N=124) had *never smoked*, Low Frequency Smokers (N=107) *smoke once in a while, but not every day* or *have smoked 1 or 2* *cigarettes ever* and High Frequency Smokers (N=38) *smoke every day*. Participants who *used to smoke, but have quit* were excluded from the analysis (N=6).

Table 4.1. History of alcohol use descriptive variables.

		Frequency of occurrence (%)				
Frequency of alcohol use (times used)	N	Never	1-5	5-10	10-20	>20
How often have you used alcohol						
in your lifetime?	268	8.2	10.8	9.3	10.8	60.8
in the past 12 months?	268	13.1	14.2	19.0	14.2	39.6
in the past 4 weeks?	268	23.1	43.7	21.6	6.7	4.9
How often have you been drunk or tipsy						
in your lifetime?	271	31.7	40.6	9.2	7.0	11.4
in the past 12 months?	267	39.7	42.7	7.5	4.1	6.0
in the past 4 weeks?	266	66.2	30.1	3.4	0.4	0.0
	N	**Age (years)**				
Begin alcohol use		never	< 9	10-12	13-15	>16
How old were you the first time you						
drank at least one glass of alcohol?	234		1.3	18.4	69.7	10.7
were drunk?	266	44.7	0.0	1.5	35.0	18.8

Note. <9= 9 years or younger; >16=16 years or older

Heart rate

Heart rate was measured using a three-lead electrocardiogram (ECG) and was 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. For purposes of the analyses, the stress procedure was consolidated into three periods: a pre-task rest period (Rest), the period during any of the three stress tasks that elicited the maximum HR response (Task), and a post-task recovery period (Recovery). As expected, the maximum HR response occurred for most participants during either the mental arithmetic task (33.8%) or the speech part of the public speaking task (49.8%).

Perceived stress

Self-reported PS (Dieleman, et al., 2010) was assessed after the rest period, each of the tasks and at the end of the procedure. Participants answered seven questions (e.g. 'Can you feel your heart beating?', 'Are you nervous?') using a visual thermometer ranging from 0 (not at all) to 8 (very much). The scores were summed to a total score of PS for each period/task, Task PS entailed the maximum PS score during any of the three stress tasks.

Potential covariates

In previous studies examining heart rate reactivity, age (Phillips, et al., 2009), gender (Back, et al., 2008), pubertal stage (Carroll, Phillips, & Der, 2008; Ortiz & Raine, 2004), body mass index (BMI; Carroll, et al., 2008), oral contraceptive (OC) use (Girdler, et al., 1997), socioeconomic status (SES; Miller, et al., 2009), internalizing and externalizing problems (Greaves-Lord, et al., 2007; Ortiz & Raine, 2004), parental substance use (Finn, et al., 1992) and time of test session (Sheffield, et al., 1997) have been taken into account. We assessed pubertal stage using self-reported Tanner stages (Marshall & Tanner, 1970). SES was based on the higher occupational level of either parent (Statistics, 2010) and coded into low (x=1), average (x=2) and high (x=3) SES. Internalizing and externalizing problems were evaluated using the Youth Self-Report (YSR; Achenbach & Rescorla, 2001). Scores on subscales affective, anxiety and somatic disorders were summed, leading to number of internalizing problems. Number of externalizing problems was similarly achieved (using subscales attention deficit hyperactive, conduct and oppositional defiant disorders subscales). Parent substance use was self-reported usually by the mother and entails the average number of alcoholic drinks consumed per week. Age, gender (boy: x=1; girl: x=2) and OC use (no: x=0;

yes: x=1) were assessed using a demographics self-report questionnaire. Height and weight were measured prior to the test session to calculate BMI. Time of test session was coded noon (x=1) or late afternoon (x=2).

Statistical analysis

First, a manipulation check was performed by way of repeated measures analysis of variance (RM-ANOVA) in the entire sample in order to confirm that the stressful tasks did induce an increase in HR and PS as compared to the Rest period. Age and gender were entered into all models as covariates. Prior to the main analysis additional covariates were examined and added to the main analysis if they correlated significantly with both independent and dependent variables. To investigate HR during the stress procedure, a 3x3x3 RM-ANOVA was performed with period (Rest, Task, Recovery) as the within-subjects factor and gNDW (Low, Medium, High Quantity) and gFTU (Non-smokers, Low, High Frequency Smokers) as between-subjects factors. Interactions between period and the between-subjects variables as well as between period and the covariates were examined. Simple contrasts were performed in order to explore between-group differences. Univariate ANOVAs of the change score in HR between Rest and Task periods were performed when interaction effects were present. An identical analysis was performed with PS as the dependent variable measured across the three periods. In all analyses, Greenhouse-Geisser statistics are reported when necessary to correct departures from sphericity.

RESULTS

Manipulation check

For the entire sample, the tasks produced physiological stress, as indicated by a significant within-groups effect of period ($F(1.32,357.37)=589.66$, $p<.001$). Simple contrasts showed that average HR during the Task was significantly higher than during Rest ($F(1,271)=412.99$, $p<.001$). PS also differed across the three periods ($F(1.51,414.42)=403.01$, $p<.001$), with simple contrasts again showing PS to be higher during the Task as compared to Rest ($F(1,274)=293.39$, $p<.001$).

Preliminary analyses

Descriptives of, and correlations between, dependent and independent variables and potential covariates are shown in Tables 4.2 and 4.3. Age and gender were controlled in all models. No other variables correlated with both dependent and independent variables, and therefore were not included as covariates in the models. PS and HR showed a small, significant positive correlation, specifically during Task ($R=.13$, $p<.05$) and Recovery ($R=.12$, $p<.05$), additionally PS Rest with HR Task ($R=.13$, $p<.05$) and HR Recovery ($R=.16$, $p<.01$). The HR response measure was not significantly correlated with the PS response measure.

Alcohol and tobacco use: heart rate

A significant between-subjects effect was evident for gNDW ($F(2,244)=6.12$, $p<.01$). Simple contrasts showed that Low Quantity Drinkers had a significantly higher HR during the entire procedure compared to Medium Quantity (adjusted $p<.001$) and High Quantity Drinkers (adjusted $p<.01$). Medium Quantity Drinkers did not differ significantly from High Quantity Drinkers, as shown in Table 4.4. No interaction between period and gNDW was evident.

Table 4.2. Descriptive statistics for independent and dependent variables and covariates

Variable	N	Mean (Sd)	Range	Frequency (%)
Age	275	17.22 (1.31)	14.52-20.84	
Gender (boys/girls)	275			42.5/57.5
Body mass index	273	21.99 (3.30)	16.00-36.75	
Pubertal stage	254	4.31 (0.66)	2.50-5.00	
SES (low/average/high)	274			4.4/50.0/45.6
Internalizing symptoms	243	0.93 (0.67)	0.00-3.72	
Externalizing symptoms	264	1.45 (0.70)	0.12-3.63	
Parental alcohol use	247	5.22 (6.71)	0.00-42.00	
Time test (noon/late afternoon)	274			59.9/40.1
Oral contraceptive use (use/no use)	155			54.8/45.2
gNDW (low/med./high quantity)	275			37.5/34.5/28.0

Table 4.2 continued.

Variable	N	Mean (Sd)	Range	Frequency (%)
gFTU (non-smokers/low/high freq.)	269			46.1/39.8/14.1
Heart rate (bpm) during	275			
Rest		75.87 (9.99)	55.62-122.43	
Task		86.33 (12.34)	60.04-141.16	
Recovery		71.92 (9.06)	54.03-102.81	
Perceived stress during	275			
Rest		7.88 (5.95)	0-32	
Task		14.92 (8.93)	0-42	
Recovery		3.89 (4.98)	0-34	
Drinks per week	275	5.36 (5.80)	0-39	
Low quantity drinkers		0.68 (0.82)	0-2	
Med. quantity drinkers		4.56 (1.19)	3-6	
High quantity drinkers		12.59 (5.94)	7-39	
Number of cigarettes per day*	38	10.97 (6.08)	2-30	

* pertains to high frequency smokers only.

Note. SES=socioeconomic status; gNDW=group of Number of Drinks per Week; gFTU=group of Frequency of Tobacco Use; med.=medium; freq.=frequency; bpm= beat per minute.

There was no main effect of period, nor a between-subjects effect for gFTU in the RM-ANOVA. However, an interaction effect of gFTU with period was evident ($F(2.62,319.57)=5.54$, $p<.01$). A three-way interaction effect between gNDW, gFTU and HR was not. To investigate the interaction between gFTU and period, a univariate analysis of change scores in HR from Rest to Task was performed and revealed a significant between-subjects effect ($F(2,258)=10.42$, $p<.001$), with simple contrasts showing that High Frequency Smokers portrayed blunted HR reactivity to the tasks as compared to Low frequency smokers (adjusted *p*<.001) and Non-smokers (adjusted *p*<.001). Low frequency smokers did not differ significantly from Non-smokers. Results are depicted in Figures 4.3 (gNDW) and 4.4 (gFTU).

Alcohol and tobacco use: perceived stress

Predicting PS, a within-subjects effect of period over time was evident (F(1.51,389.12)=7.66, *p*<.01). No between-subjects effects of gNDW or gFTU or interactions effects were observed.

Covariates

In order to examine whether the found effects were specific to alcohol and tobacco use alone, number of externalizing problems was added to the model predicting HR. The interaction between number of externalizing problems and HR was not significant, and the results of the model did not change.

In the model predicting HR, there was no main effect of gender, though an interaction effect of period and gender was found (F(1.31,319.57)=4.57, *p* <.05). A univariate ANOVA analysis showed this interaction to be due to girls reacting more strongly to the stress procedure; the change in HR from Rest to Task was greater for girls than for boys (F(1,273)=4.06, *p*<.05). No main or interaction effect of gender was significant in the model predicting PS. When both RM-ANOVAs were run again in female subjects only, controlling for OC use, there were no main or interaction effects of OC use.

Table 4.3. Correlations between independent and dependent variables and covariates.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.HR Rest	1																	
2.HR Task	**.68**	1																
3.HR Rec	**.92**	**.76**	1															
4. gNDW	**-.25**	**-.23**	**-.29**	1														
5. gFTU	-.08	**-.21**	*-.16*	**.38**	1													
6. PS Rest	.10	*.13*	**.16**	-.04	-.08	1												
7. PS Task	.04	*.13*	.09	-.02	-.10	**.65**	1											
8. PS Rec	.07	.10	*.12*	-.03	-.03	**.68**	**.49**	1										
9. Age	.07	.03	.04	**.22**	*.16*	-.10	-.11	-.00	1									
10.Gender	.06	*.15*	.03	*-.14*	*.13*	*-.13*	-.04	-.07	.04	1								
11.BMI	-.05	-.11	-.02	.05	**.17**	-.05	-.06	.02	.07	*.15*	1							
12.Puberty	**.20**	.08	*.15*	.07	.09	.02	-.02	.00	**.32**	-.04	**.22**	1						
13.SES	-.10	.07	-.09	-.03	-.06	.02	.01	-.02	-.04	.11	*-.13*	-.05	1					
14.Intern	.09	.08	*.13*	-.03	.12	-.01	*.16*	.03	.03	**.39**	.04	.03	-.08	1				

15.Extern	*-.13*	*-.14*	*-.15*	**.27**	**.26**	-.02	.01	-.01	-.03	-.04	.01	.04	**-.17**	**.35**	1			
16.PAU	.05	.04	-.02	.12	.07	.07	.05	.03	.05	.02	-.08	-.03	**.22**	-.01	-.08	1		
17.TTS	-.02	.06	.07	-.10	.01	-.12	-.08	-.04	-.03	.06	-.10	-.00	.05	.05	-.03	-.06	1	
18.OC use	.10	-.03	.10	**.23**	**.36**	-.06	-.06	-.15	**.29**	Na	.01	**.29**	-.07	.08	-.01	.03	.00	1

Italics: $p<.05$; **bold**: $p<.01$

Note. HR = heart rate; Rec = recovery; gNDW = group of Number of Drinks per Week; gFTU = group of Frequency of Tobacco Use; PS = perceived stress; BMI = body mass index; Puberty = pubertal stage; SES = socioeconomic status; Intern = internalizing; Extern = externalizing; PAU = parent alcohol use; TTS = time of test session; OC = oral contraceptive; Na = not applicable, as OC use was only present in girls

Table 4.4. Values for repeated measures ANOVA model.

Variable	F	*p*	η^2
Period	2.59	.10	.01
Between-subjects effects			
gNDW	6.12	<.01	.05
gFTU	0.61	.55	.01
Age	3.24	.07	.01
Gender	1.28	.26	.01
Interaction effects			
Period * gNDW	0.15	.96	.00
Period * gFTU	5.54	<.01	.04
Period * Age	0.16	.75	.00
Period * Gender	4.57	<.05	.02
Period * Externalizing	0.11	.81	.00
Period * gNDW * gFTU	0.69	.64	.01
Variable	Contrast estimate	*p*	
Contrast analysis gNDW			
Low vs. Med. Quantity Drinkers	6.08	<.001	
Low vs. High Quantity Drinkers	5.84	<.01	
Med. vs. High Quantity Drinkers	-0.23	.89	

Note. All values are from the repeated measures ANOVA with number of externalizing symptoms included; N=256; gNDW = group of Number of Drinks per Week; gFTU = group of Frequency of Tobacco Use; Med. = Medium

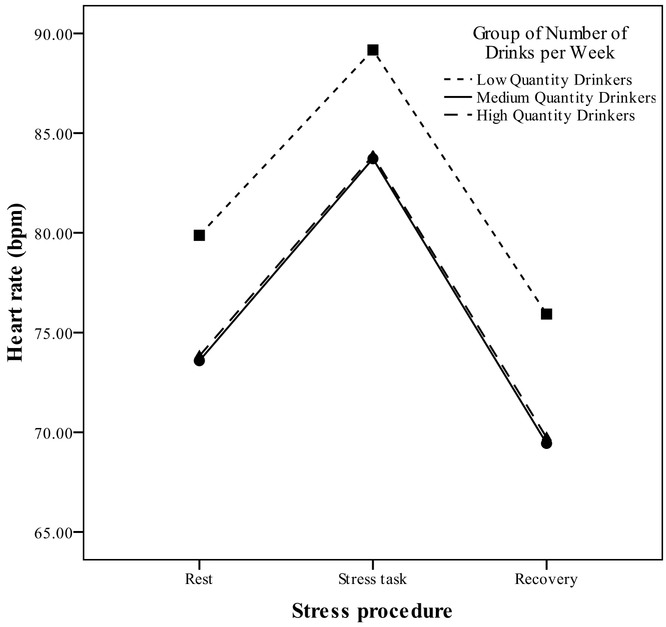

Figure 4.3. Heart rate during the stress procedure for each group of low, medium and high quantity drinkers, corrected for age, gender and externalizing problems.

Note: covariates appearing in the model are evaluated at the following values: age=17.21, gender=0.5, number of externalizing problems=-.01.

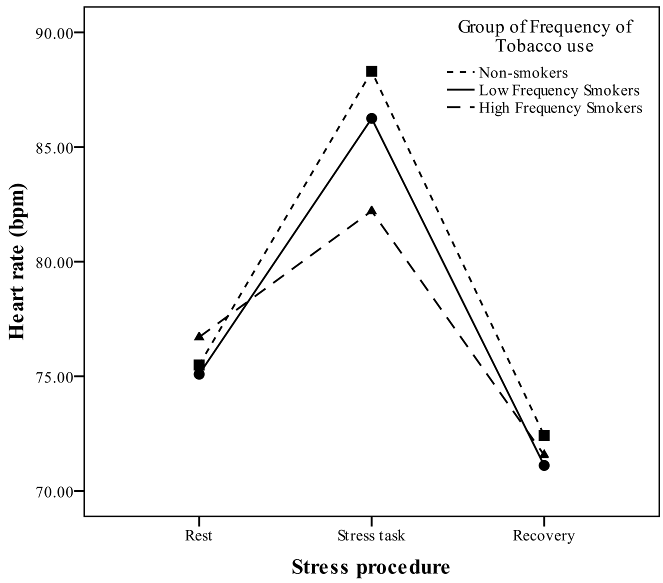

Figure 4.4. Heart rate during the stress procedure for each group of non-smokers, low and high frequency smokers, corrected for age, gender and externalizing problems.

Note: covariates appearing in the model are evaluated at the following values: age=17.21, gender=0.59, number of externalizing problems=-.01.

DISCUSSION

In a sample of 14- to 20-year old adolescents, we examined whether alcohol and tobacco use were related to heart rate (HR) during a psychosocial stress procedure. To our knowledge, this is the first study to examine autonomic nervous system (ANS) (re)activity in relation to substance use in adolescents from the general population. We found that those who drank a medium and high number of alcoholic drinks per week (more than 2) portrayed a lower HR during the entire stress procedure as compared to those who drank fewer alcoholic drinks per week. Also, those who used tobacco every day showed blunted HR reactivity to the stressful tasks as compared to those who smoked less frequently or not at all. Thus, two of our hypotheses were confirmed (i.e. adolescents who drank more alcohol portrayed lower resting HR, and those who used more tobacco portrayed blunted HR response to stress) and two were not (i.e. we expected to find that those who drank more alcohol would portray blunted HR response to stress, and those who used more tobacco would portray lower resting HR). Furthermore, we examined whether HR responses were related to PS responses, and whether the groups of alcohol and tobacco users differed with respect to PS responses. As expected, HR and PS responses were positively and significantly correlated, and PS responses were not related to alcohol or tobacco use.

Previous research showed that drinking more during adolescence strongly predicts the prevalence of alcohol use disorders in adulthood (Bonomo, et al., 2004). Therefore the adolescents in our sample who drank more may form a group of those at risk for later alcohol use problems. Though we were unable to examine causality in this study, these preliminary findings may provide support for the theory of an inherent hypo-arousal of the ANS in individuals more vulnerable to substance use problems. These individuals may deliberately seek out and use alcohol or tobacco in order to achieve a state of normalized arousal (Goeders, 2003; Majewska, 2002). Because this frequent use of substances as a way of seeking stimulation occurs at an early age, during adolescence, these individuals could be more vulnerable to SUDs later in life.

Interestingly, adolescents who drank medium and high quantities of alcohol per week showed a lower HR during the entire stress procedure; a between-subjects effect was evident in the analyses. However, no interaction effect was observed, as we had expected. All groups showed a relatively similar peak in HR in response to the tasks. Adolescents who drank more thus did not react physiologically differently to the stressful tasks; they portrayed a more general lowered HR. As this study was performed cross-sectionally, we are unable to differentiate whether this effect is due to underlying ANS variation, or whether use of alcohol has already affected the ANS in these adolescents. However, due to the young age of the adolescents in our sample, and that they have as yet used relatively little alcohol, we consider it unlikely that the observed differences of the ANS are due to the use of alcohol and thus are possibly due to an underlying difference in general ANS regulation. Our results suggest additionally that this difference may not lie in the immediate *response* to stress, rather in overall ANS activity across situations. The present results are supported by our previously reported findings, in the same sample, of lower hypothalamus-pituitary-adrenal (HPA) axis activity (indexed by salivary cortisol) during the Rest and Task period in adolescents who began drinking at an earlier age (Evans, et al., 2012). HPA and ANS measures in this sample were related, as indicated by significant positive correlations between cortisol and HR during Rest ($R=.18$, $p<.01$) and Task ($R=.32$, $p<.001$), as well as the response measures ($R=.16$, $p<.05$), thus reinforcing our findings of low physiological arousal in those more prone to risky substance use. These findings are in line with earlier suggestions that physiological stress response dysregulation in adolescents may signal vulnerability to various kinds of psychopathology (Stroud, et al., 2009).

This is the first study to examine the relation between alcohol use and HR in a general adolescent population, therefore, the results are preliminary and must be interpreted cautiously. Our finding that those who drank more portrayed a lower HR during the stress procedure is in line with one finding in adults with a FH of alcoholism (Sorocco, et al., 2006), though in contrast to other similar studies which found increased HR in response to unavoidable shock (Finn, et al., 1992; Finn & Pihl, 1987) and a mental arithmetic task (Harden & Pihl, 1995). Further research in this area is needed in order to clarify these contrasting findings.

We observed that PS was significantly and positively related to HR, which confirmed findings from a previous study in adolescents from the general population (Oldehinkel, et al., 2011). We did not find a relation between PS and alcohol and tobacco use, corroborating earlier reports of no difference in PS between control subjects and those at risk for a SUD (Finn & Pihl, 1987) and those exhibiting more externalizing problems (Fairchild, et al., 2008). This was in line with our expectations; physiological responses reflect underlying, biological processes, and we would not necessarily expect similar relations to be found with the subjective experience of a stressor. Physiological and perceived stress are distinct constructs (Oldehinkel, et al., 2011), which was substantiated in our finding of a significant and positive, but not strong, correlation between HR and PS.

Our observations indicate a relation between tobacco use and HR reactivity. Those who smoked every day showed a blunted HR response to the stressful tasks compared to those who smoked less frequently or not at all. This finding is in line with several findings on adult smokers (Girdler, et al., 1997; Phillips, et al., 2009; Roy, et al., 1994; Sheffield, et al., 1997; Straneva, et al., 2000) though is in contrast to other studies (Back, et al., 2008; Childs & de Wit, 2009; Hughes & Higgins, 2010; Kirschbaum, et al., 1993; Perkins, et al., 1992;

Tersman, et al., 1991). While two studies examining HR reactivity in low versus high frequency tobacco users found no difference between these groups (both portrayed attenuated responses), we found that adolescents who smoked less frequently did not differ significantly from those who had never smoked. It is possible that in adolescents, underlying variation of the ANS is only evident in those who use tobacco more frequently.

Attenuated HR during rest as well as in reaction to stress in those who exhibit more externalizing problems has been well established (Ortiz & Raine, 2004), as has the relation between externalizing problems and substance use (Krueger, et al., 2002; Young, Stallings, Corley, Krauter, & Hewitt, 2000; Zimmermann, Blomeyer, Laucht, & Mann, 2007). Therefore, we examined whether hypo-arousal of the ANS was specific to substance use, as opposed to generally related to externalizing problems. When number of externalizing problems was controlled for in the model, the relations between HR and alcohol and tobacco use remained significant, thus providing evidence for a relation between HR hypo-arousal and substance use specifically.

The time at which the last cigarette was smoked prior to the stress procedure was not asked, thus it cannot be excluded that the observed blunted HR reactivity to stress was due to nicotine withdrawal; smokers may have been less able to concentrate and were therefore perhaps less able to engage in the stressful tasks (Phillips, et al., 2009). However, in two studies, nicotine withdrawal did not influence the response to stress (al'Absi, et al., 2003; Tsuda, et al., 1996). Furthermore, smokers exhibited a blunted reaction to stress whether they did or did not wear a nicotine patch (Girdler, et al., 1997). Moreover, it is unlikely that cigarettes that may have been smoked just prior to the session influenced HR during the stress procedure due to the fact that nicotine causes an increase in HR (Hasenfratz & Battig, 1992; James & Richardson, 1991; Pauli, et al., 1993) while we observed no differences in pre-task HR in High Frequency Smokers as compared to Non- and Low Frequency Smokers.

With regard to this study, the following should be taken into account. As mentioned above, the nature of the study was cross-sectional. We are unable to exclude the possibility of alcohol and tobacco use affecting the ANS directly. While we believe it to be unlikely because they have used relatively little alcohol and tobacco, this remains to be elucidated. Secondly, possible effects of a third variable cannot be eliminated. It is viable that factors such as temperament or the experience of stressful events in the past influenced the relation between alcohol and tobacco use and physiological stress (re)activity in this study. Thirdly, we had no information regarding the time at which the last cigarette was smoked, and therefore cannot exclude possible direct effects of nicotine or nicotine withdrawal on HR. Fourthly, we based our alcohol use variable on third percentiles, as has been done in previous studies (Hillers & Massey, 1985; Murray, et al., 2002), in order to obtain relatively equal groups. A more widely used variable, such as binge-drinking, would be interesting, but was not possible in a general population group of this size and of this age group in which most adolescents have not yet begun using large amounts of alcohol. Future follow-up measurements of this group will allow a more comprehensive investigation of risky substance use in relation to physiological stress (re)activity.

In conclusion, lower HR during a psychosocial stress procedure was evident in those adolescents from the general population who drank more as compared to those who drank less. Also, adolescents who smoked every day showed a blunted HR response to the stressful tasks as compared to those who smoked less frequently or not at all. PS was significantly correlated with HR, and was not related to alcohol or tobacco use. Though data are preliminary and must be interpreted with caution, it is possible that adolescents prone to use more alcohol and tobacco may be characterized by hypo-arousal. Conceivably, these adolescents use substances in order to seek stimulation and achieve a more normalized state of arousal. As they use more alcohol and tobacco, they may be vulnerable to SUDs later in life. ANS activity during rest as well as in reaction to stress may thus be a potential vulnerability marker for SUDs in adolescents.

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