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Reduced autonomic flexibility as a predictor for future anxiety in girls from the general population: The TRAILS study

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ABSTRACT

The present study investigated whether autonomic flexibility predicted future anxiety levels in adolescent boys and girls. This study is part of the TRacking Adolescents' Individual Lives Survey (TRAILS), a prospective cohort study of Dutch adolescents. The current study included a subsample of 965 individuals. Measures of autonomic flexibility, i.e., heart rate (HR) and respiratory sinus arrhythmia (RSA), were determined during the first assessment wave (T1: participants 10–12 years old). Self-reported anxiety was assessed at the first and second assessment wave (T2: participants 12–14 years old). Possible gender differences and co-occurring depressive problems were examined. In girls, low RSA predicted anxiety levels 2 years later. In boys, no associations between HR and RSA and future anxiety were found. We conclude that in adolescent girls from the general population, signs of reduced autonomic flexibility (i.e., low RSA) predict future anxiety levels. Since the effect size was small, at this point, RSA reactivity alone cannot be used to identify individuals at risk for anxiety, but should be regarded as one factor within a large group of risk factors. However, if the present findings are replicated in clinical studies, intervention programmes – in the future – aimed at normalising autonomic functioning may be helpful.

1. Introduction

The onset of adolescence is characterised by high levels of anxiety, especially in girls (Verhulst et al., 1997). Since anxiety problems put forth an enormous burden in both human and economic terms, it is important to investigate putative indicators of future anxiety in early adolescence.

One factor that may indicate vulnerability towards anxiety is reduced autonomic flexibility (Friedman and Thayer, 1998a,b; Friedman, 2007). An autonomic nervous system (ANS) that lacks flexibility may hinder an individual in adequately responding to a constantly changing environment. This may enhance feelings of anxiety and lack of control, and thus may increase the risk for anxiety problems.

Autonomic flexibility is reflected in cardiovascular measures, such as heart rate (HR) reactivity. Furthermore, indices such as respiratory sinus arrhythmia (RSA) provide more precise information on autonomic flexibility, since cardiovascular functions are influenced both by sympathetic and parasympathetic activity, and RSA more specifically reflects parasympathetic activity (Mezzacappa et al., 1997). It is important to investigate measures of parasympathetic activation such as RSA when investigating the relation between autonomic flexibility and anxiety, since Porges (1995, 2001) proposed that some individuals may be at risk for developing various mental and physical health problems, among which anxiety, due to decreased parasympathetic activation.

RSA can be determined by performing power spectral analyses on the HR signal. This generates measures of HR variability (HRV). HRV in the high-frequency band (0.15–0.40 Hz) is often called RSA (e.g., Akselrod et al., 1981) since it is related to respiratory variations in HRV. HR and RSA are commonly assessed during rest (i.e., baseline), and in reaction to a physical or psychological/mental stressor, so that HR or RSA *r*eactivity can be determined by calculating the difference between the two. HR and RSA reactivity can be used as indices of autonomic flexibility.

Previous studies on the relation between autonomic flexibility and anxiety are equivocal, showing evidence for either higher or lower autonomic flexibility in relation to anxiety. For instance, in cross-sectional studies of adults with anxiety problems, evidence was found for low HRV

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reactivity, reflecting lower autonomic flexibility (Friedman et al., 1993; Klein et al., 1995; Piccirillo et al., 1997; Tulen et al., 1996). However, the few findings in adolescents are less clear cut. Gerra et al. (2000) found that adolescent boys with an anxiety disorder showed a higher HR reactivity to a mixed-model stress task than controls. This suggests increased autonomic flexibility. Further, Mezzacappa et al. (1997) found a relation between anxiety and higher baseline HR when performing an orthostatic challenge test in adolescent males. This finding of a higher baseline HR could indicate reduced autonomic flexibility, when interpreting the already- high baseline HR values as ceiling effects. However, this explanation does not apply to the finding of higher HR *r*eactivity in relation to anxiety (Gerra et al., 2000). To elucidate the relation between autonomic flexibility and anxiety in adolescence, it is therefore important to investigate measures of both baseline autonomic activity and reactivity.

In our earlier studies we investigated baseline and reactivity measures of HR and RSA in a general population sample aged 10–13 years old (Dietrich et al., 2007; Greaves-Lord et al., 2007). Since previous studies mainly concerned male adolescents, and did not consider the often co-occurring symptoms of depression, we also took into account these factors. We found that internalising problems (i.e., anxiety, depression and somatic complaints) were associated with higher baseline HR and lower baseline RSA (Dietrich et al., 2007), and more specifically, that anxiety was related to lower baseline RSA, especially in boys (Greaves-Lord et al., 2007).

Our previous work only concerned cross-sectional data. In a cross-sectional design it remains unclear whether autonomic flexibility indeed results in anxiety, or whether autonomic flexibility is in fact the result of long periods of anxiety. Therefore, a longitudinal design is needed to consider the predictive value of autonomic measures for future anxiety. To our knowledge, prospective studies on the relationship of autonomic flexibility with future anxiety levels in young adolescents are lacking. Therefore, the aim of the present study was to investigate whether measures of autonomic flexibility predict future anxiety levels, using data from a large, prospective cohort study of both boys and girls. We assessed HR and RSA in rest, and in reaction to an orthostatic challenge test. Possible gender differences and the role of co-occurring depressive problems were also examined.

2. Methods

2.1. Sample and procedure

The present study was part of the TRacking Adolescents' Individual Lives Survey (TRAILS). TRAILS is a prospective cohort study of Dutch young adolescents, who were 10–13 years old at the first assessment wave (wave 1; 2001–02; extensive study of a) psychopathology, including anxiety, and b) putative risk factors, including autonomic measures). They were re-assessed 2 years later in 2003–04 (wave 2; re-assessment of psychopathology, including anxiety). The target sample consisted of adolescents from five municipalities in the North of The Netherlands, including both urban and rural areas. Of all eligible individuals ($n=2935$), 76.0% participated in the study ($n=2230$, mean age 11.09 years, S.D. 0.55, 50.8% girls). Participants did not differ from those who refused with respect to the proportion of single-parent families, the prevalence of teacher-rated problem behaviour, several sociodemographic variables and mental health outcomes (De Winter et al., 2005). At wave 2, information was obtained from 2149 (96.4%) of those who participated at wave 1 (mean age 13.56 years, S.D. 0.53, 51.0% girls). For the current manuscript, we included a subsample of 965 boys and girls (47% vs. 53%, mean age 11.0 years, S.D. = 0.51) from whom complete data concerning autonomic flexibility (wave 1) and anxiety (wave 2) were available. To examine possible selection effects, a logistic regression analysis was performed with 'complete data yes/no' as the dependent variable and gender, pubertal stage, socioeconomic status and wave 1 anxiety scores as predictors. The 965 participants with complete data did not differ from the other 1265 participants in the TRAILS study regarding gender, pubertal stage and wave 1 anxiety scores. The current subsample had a somewhat higher socioeconomic status; however, the effect size was small (Cox and Snell $R^2=0.7\%$). Written consent was obtained from the children's parents. The study was approved by the Central Dutch Medical Ethics Committee.

2.2. Measures

2.2.1. Anxiety and co-occurring depressive problems

Anxiety levels were assessed using a self-report questionnaire: the Revised Child Anxiety and Depression Scale (RCADS; e.g. Chorpita et al., 2000). The RCADS assesses anxiety and depressive symptoms thoroughly; it contains 47 items that are scored on a four-point scale (0 = never, 1 = sometimes, 2 = often, 3 = always). The questionnaire covers several dimensions of anxiety that closely correspond to the Diagnostic and Statistical Manual of Mental Disorders (DSM) clinical diagnoses of anxiety disorder, that is, separation anxiety disorder, generalised anxiety disorder, social phobia, panic disorder and obsessive–compulsive disorder. In addition, it assesses depressive symptoms that are closely related to the diagnosis of major depressive disorder. In this study, anxiety scores (i.e., the mean scores on all anxiety items) were computed by summing the scores on all anxiety items and dividing this by the number of items that was completed. Similarly, depression scores were computed. The Cronbach's alphas based on the present data set were – respectively – at wave 1/wave 2 — 0.91/0.93 for the anxiety scale, and 0.72/0.81 for the depression scale.

2.2.2. Autonomic flexibility

2.2.2.1. Baseline HR and RSA. Measures of cardiovascular function (i.e., HR) were performed in a quiet room at school, one person at a time. Participants were encouraged to relax, and were told to breath spontaneously, and not to move or speak during data acquisition. Subsequently, the procedure was explained to them. A three-lead electrocardiogram (ECG) was recorded to register HR. The recordings did not start until the participant had been in supine position for approximately 5 min, and the signal was stabilised. ECG signals were registered for 4 min while the participant was in supine rest.

2.2.2.2. HR and RSA reactivity. An orthostatic challenge test (i.e., natural standing action) was used to provoke ANS activation. The orthostatic challenge test is a widely used standardised physical test to assess autonomic reactivity to the act of standing. Typically, the standing action provokes an increase in cardiac activity, that is, it elevates HR and reduces RSA. In this test, the psychological, motivational, and cognitive processes that play a role in mental stress tasks do not intervene. After the ECG registration in supine rest, the participant was asked to actively stand up. Again, after signals were stabilised in the upright position, the ECG signal was registered for another 2 min to assess autonomic reactivity to the act of standing.

2.2.2.3. Spectral analyses. HR recordings were digitised using a DAS-12 data acquisition card for notebooks (Keithley Instruments, Cleveland, OH, USA) and stored on hard disk for off-line analysis. The sample rate was 100 Hz. R. A special interpolation algorithm was used that increased the time resolution for *R*-peak detection by a factor of 2.5. This resulted in interbeat-intervals (= the intervals of time between two consecutive heart beats i.e., *R*-peaks, IBI's) with sufficient resolution for HRV determination (see Appendix A for further information on this procedure). The analysed time series were checked for stationarity and corrected for artefacts. HR was calculated as 60 000/mean IBI and expressed in beats per minute (bpm). HRV power in the high-frequency band (0.15–0.40 Hz), that is, RSA, was then computed by means of power spectral analysis (CARSPAN software program; Mulder et al., 1988; Van Steenis et al., 1994). CARSPAN allows for the discrete Fourier transformation (DFT) of non-equidistant IBI-series. The DFT is applied to a time series that consists of a series of Dirac function at the time points of *R*-peaks. The spectral contribution of each Dirac pulse can be easily calculated and the amplitude spectrum is the summation of the spectral contributions of the pulses. In this way, the limitation of the fast Fourier transformation (i.e., the number of points included must be a power of 2) does not apply, and time series of any segment length can be transformed. More detailed information has been reported elsewhere (Dietrich et al., 2006).

To approximate a normal distribution, HR and RSA were transformed to their natural logarithm and centred. Reactivity measures were calculated by subtracting the values obtained during supine rest (i.e., baseline) from the measures obtained during active standing.

2.2.3. Other individual characteristics

Some variables, such as pubertal stage, might be associated with both anxiety and autonomic functioning, and could play a role in the relation between the two. Therefore, a large number of possible covariates were assessed in the TRAILS study. In the current sample, only pubertal stage and gender were associated with both anxiety and autonomic functioning. Effects of gender were investigated thoroughly by specifically examining gender differences (i.e., moderating effects).

Furthermore, pubertal stage was taken into account as a covariate. Information on pubertal stage was obtained at wave 1. Pubertal stage was assessed using schematic drawings of secondary sex characteristics associated with the five standard Tanner stages of pubertal development, yielding a categorical variable of stages 1–5 (Marshall and Tanner, 1969, 1970). The parent – usually the mother – was provided with gender-appropriate sketches, and asked which of the sketches looked most like their child. These ratings have been widely used and have demonstrated good reliability and validity (e.g., Dorn et al., 1990).

Other possible covariates, such as disruptive behaviour and body mass index (BMI) were also assessed, but were not taken into account in the present study, because these factors were not associated with both anxiety and autonomic functioning, and did not markedly influence the relationship between the two in the current data set.

2.3. Statistical analyses

2.3.1. Autonomic reactions

Paired-samples *t*-tests were computed comparing HR and RSA in supine position with HR and RSA in standing position, to investigate whether the orthostatic challenge

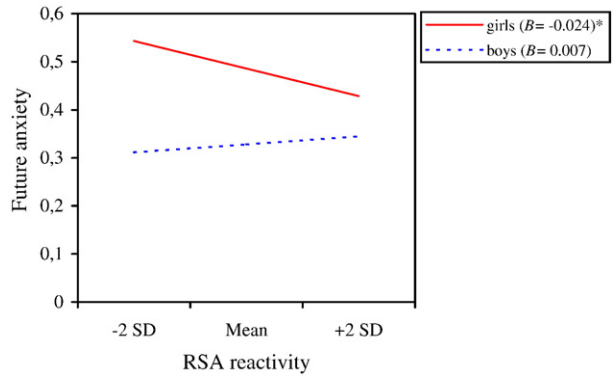

Fig. 1. Gender differences in the relationship between RSA reactivity measures and anxiety scores two years later. *Note*: RSA = respiratory sinus arrhythmia, reactivity = difference between measures in supine and standing position, S.D. = standard deviation, *B* = beta; unstandardised regression coefficient, i.e. simple slope, * = significant slope ($P<.05$).

task provoked a significant reaction in the expected direction (i.e., increase in HR, decrease in RSA).

2.3.2. Descriptives

Means, standard deviations and ranges were computed for all variables.

2.3.3. Multiple regression analyses

To investigate whether HR and RSA (baseline and reactivity) predicted future anxiety levels, four regression analyses were performed with wave 2 anxiety scores as the dependent variable. In each set of analyses, the autonomic measure (HR or RSA, either baseline or reactivity) was added as the predictor in the first block (model 1). In the second block, gender was added (coded 0 = girl, and 1 = boy; model 2). In the third block (model 3), the interaction between gender and the respective ANS measure was added to investigate possible gender differences. Subsequently, Tanner stage was added to investigate the effects of pubertal stage (model 4). In the fifth block (model 5), wave 1 anxiety scores were added to control for cross-sectional effects of anxiety, and check the prospective nature of the associations tested. Lastly, to investigate whether associations were specific for anxiety, as apart from depression, depression scores were added in the last two blocks: first wave 1 depression was added (model 6) and finally wave 2 depression (model 7).

To check for multicollinearity, correlations between predictors were calculated. All correlations were below 0.90, with the highest correlation being 0.66. Thus, there was no sign of multicollinearity, and it was possible to include all predictors in the models.

2.3.4. Post hoc testing

In case of a significant effect of an interaction term between gender and an autonomic measure, additional *post hoc* analyses were performed. To further investigate gender differences similar multiple regression analyses were performed, however, stratified for gender, thus leaving out the main effect of gender, and the interaction term between gender and the autonomic measure. In order to calculate beta's that could be interpreted in such a way that they could be used to construct illustrative figures, *post hoc* probing tests were performed (Holmbeck, 2002). In these analyses, main effects of the autonomic measures (i.e., the unstandardised beta's) in boys were calculated by coding gender as: boys = 0, girls = −1, and main effects of the autonomic measures (i.e., the unstandardised beta's) in girls were investigated by coding gender as: girls = 0, boys = 1. Since autonomic measures were centred (i.e., mean = 0), the unstandardised beta's could be interpreted as simple slopes, and from this, Fig. 1 was constructed.

Second, since autonomic reactivity may be diminished because of already-high high baseline HR values (ceiling effects) or already-low baseline RSA values (floor effects), we performed *post hoc* analyses for the HR and RSA reactivity measures in which we controlled for the baseline HR or RSA (i.e., similar multiple regression analyses, however adding the baseline measure as a last step, in model 8).

In all analyses, a *P*-value smaller than 0.05 was considered statistically significant. Since we performed several statistical tests, the results may suffer from capitalisation on chance: one would expect some 5% of the associations examined to be significant merely on the basis of chance. Hence, a statistically significant result in this context does not have the same weight as significant results in a classical experimental design.

3. Results

3.1. Autonomic reactions

Both HR and RSA changed significantly in reaction to the orthostatic challenge test (change from supine rest to active standing; HR: $t = -57.5$, $df = 964$, $P<0.01$, RSA: $t = 36.4$, $df = 964$, $P<0.01$). As expected, HR increased significantly, whereas RSA decreased significantly in reaction to standing up.

3.2. Descriptives

Mean scores, standard deviations and ranges of all independent and dependent variables are presented in Table 1.

3.3. Multiple regression analyses

Table 2 shows results of the two linear regression analyses performed with wave 2 anxiety scores as the dependent variable, and the separate *baseline* autonomic measures, HR and RSA, as the predictors. Table 3 shows the results regarding the autonomic *reactivity* measures.

3.3.1. Baseline HR and RSA

None of the baseline ANS measures at supine rest had a significant main effect on wave 2 anxiety scores (model 1). Moreover, no interaction effects with gender were found (model 2).

3.3.2. HR and RSA reactivity

There were no main effects of HR and RSA reactivity (model 1). However, the interaction term between gender and RSA reactivity predicted wave 2 anxiety scores significantly ($B = 0.038$, CI = 0.008–0.067, $P = 0.012$, $\Delta R^2 = 0.6\%$; model 3), indicating a gender difference in the association between RSA and anxiety levels two years later. This difference is discussed and illustrated below (*'Post hoc analyses'* section and Fig. 1). The effect of the interaction term gender*RSA reactivity did not remain significant after adjusting for co-occurring depressive problems (models 6 and 7), and thus was not specific for anxiety, but applied to internalising problems more generally.

Table 1
Descriptives of all autonomic measures, anxiety scores, Tanner stage, and depression scores.

Measures	Boys			Girls			Total		
	n	Mean (S.D.)	Range	*n*	Mean (S.D.)	Range	*n*	Mean (S.D.)	Range
HR baseline (bpm)	482	75.7 (10.3)	51.7–111.7	541	79.1 (11.0)	49.1–115.9	1023	77.5 (10.8)	49.1–115.9
RSA baseline (ln (ms^2))	482	7.46 (1.32)	3.11–10.47	541	7.21 (1.29)	3.01–10.55	1023	7.33 (1.31)	3.01–10.55
HR reactivity (bpm)	482	17.1 (9.2)	−3.3–52.6	541	16.4 (9.1)	−9.01–49.18	1023	16.7 (9.1)	−9.01–52.57
RSA reactivity (ln (ms^2))	482	−1.47 (1.2)	−6.35–1.79	541	−1.29 (1.2)	−5.31–2.11	1023	−1.38 (1.2)	−6.35–2.11
Anxiety scores wave 2	456	0.33 (0.24)	0.00–1.43	509	0.48 (0.30)	0.00–1.78	965	0.41 (0.28)	0.00–1.78
Anxiety scores wave 1	482	0.53 (0.32)	0.00–1.89	541	0.61 (0.33)	0.00–1.97	1023	0.57 (0.33)	0.00–1.97
Tanner stage wave 1	450	1.73 (0.56)	1–5	526	1.97 (0.82)	1–5	976	1.86 (0.72)	1–5
Depression scores wave 1	476	0.62 (0.34)	0.00–2.10	540	0.62 (0.32)	0.00–2.00	1016	0.62 (0.33)	0.00–2.10
Depression scores wave 2	456	0.33 (0.30)	0.00–1.60	509	0.47 (0.36)	0.00–1.90	965	0.41 (0.34)	0.00–1.90

Note: HR = Heart Rate, RSA = respiratory sinus arrhythmia, reactivity = difference between measures in supine and standing position.

Table 2
Regression models with baseline autonomic measures as predictors and anxiety 2 years later as the dependent.

Models/predictors		HR baseline				RSA baseline			
		B	*P*	ΔR^2	*F*(*df*)	*B*	*P*	ΔR^2	*F*(*df*)
Model 1	Main effect	0.001	0.558	<0.01	0.34 (1)	0.002	0.824	<.01	0.05(1)
Model 2	Main effect	−0.001	0.478	7.6	37.9(2)	0.007	0.328	7.7	38.1(2)
	Gender	−0.158	<0.01			−0.157	<0.01		
Model 3	Main effect	−0.001	0.367	<0.01	25.3(3)	0.015	0.114	0.2	26.0(3)
	Gender	−0.158	<0.01			−0.157	<0.01		
	ANS*gender	0.001	0.575			−0.018	0.207		
Model 4	Main effect	−0.001	0.391	0.4	19.9(4)	0.017	0.073	0.4	20.6(4)
	Gender	−0.152	<0.01			−0.152	<0.01		
	ANS*gender	0.001	0.573			−0.020	0.157		
	Tanner stage	0.024	0.058			0.027	0.036		
Model 5	Main effect	0.000	0.762	19.5	69.5(5)	0.013	0.143	19.4	70.1(5)
	Gender	−0.121	<0.01			−0.121	<0.01		
	ANS*gender	0.000	0.903			−0.011	0.388		
	Tanner stage	0.016	0.156			0.018	0.115		
	Anxiety wave 1	0.390	<0.01			0.390	<0.01		
Model 6	Main effect	0.000	0.748	0.1	58.1(6)	0.013	0.138	0.1	58.5(6)
	Gender	−0.122	<0.01			−0.123	<0.01		
	ANS*gender	0.000	0.890			−0.012	0.353		
	Tanner stage	0.016	0.155			0.018	0.113		
	Anxiety wave 1	0.370	<0.01			0.369	<0.01		
	Depression wave 1	0.030	0.364			0.030	0.358		
Model 7	Main effect	0.000	0.612	28.8	168(7)	0.011	0.106	28.7	169(7)
	Gender	−0.058	<0.01			−0.058	<0.01		
	ANS*gender	0.000	0.969			−0.009	0.355		
	Tanner stage	−0.003	0.722			−0.001	0.873		
	Anxiety wave 1	0.286	<0.01			0.285	<0.01		
	Depression wave 1	−0.165	<0.01			−0.164	<0.01		
	Depression wave 2	0.528	<0.01			0.528	<0.01		

HR = heart rate.
RSA = respiratory sinus arrhythmia.
Baseline = measure during supine position.
ANS = relevant autonomic measure, thus either HR baseline or RSA baseline.
B = beta; unstandardised regression coefficient.
P = probability, significance level.
ΔR^2 = percentage of *R* square change.
F(*df*) = *F*-value ANOVA (degrees of freedom).

3.4. Post hoc analyses

3.4.1. Gender differences

As described above, an interaction effect of gender and RSA reactivity was found, indicating that results differed between boys and girls. *Post hoc* analyses revealed that, in girls, low RSA reactivity significantly predicted anxiety levels 2 years later ($B = -0.024$, CI = −0.044 to −0.004, $P = 0.017$, $\Delta R^2 = 1.1\%$, $F = 5.33$, $df = 1$, also see Fig. 1). In boys, RSA reactivity did not significantly predicted future anxiety levels ($B = 0.012$, CI = −0.014–0.028, $P = 0.517$, $\Delta R^2 = 0.4\%$, $F = 1.54$, $df = 1$).

3.4.2. Ceiling and floor effects

When we controlled for baseline RSA in the analyses concerning RSA reactivity, the effects of the gender*RSA reactivity interaction remained significant (gender*RSA reactivity: $B = 0.032$, CI = 0.006–0.058, $P = 0.017$, $t = 2.38$; ΔR^2 for baseline RSA = 0.1%, $F = 51.1$, $df = 7$), meaning that the effect of RSA reactivity was significant, independently from baseline RSA. This means that, most likely, the results are not influenced by floor effects, i.e., already-low baseline RSA, which can also be concluded from the fact that there was no significant association between baseline RSA and wave 2 anxiety scores.

4. Discussion

In the present study, we investigated whether non-invasive cardiovascular measures indicating autonomic flexibility predicted future anxiety levels in young adolescent boys and girls. Measures of baseline autonomic activity (i.e., assessed during supine rest) did not predict anxiety levels two years later. However, low RSA reactivity predicted higher anxiety levels 2 years later in girls.

4.1. Autonomic flexibility

Friedman and Thayer (1998a,b) suggested that anxious individuals are characterised by an ANS that lacks flexibility. In the present study, evidence was found for a relation between autonomic inflexibility and future anxiety: low RSA reactivity predicted higher future anxiety levels in girls. Thus, the current findings corroborate with the theory of autonomic inflexibility in anxiety, but it only applies to girls.

Measures of baseline autonomic activity (i.e., in supine rest) did not predict future anxiety problems. Moreover, the effect of RSA reactivity in girls remained significant after controlling for baseline activity. Thus, it is likely that there is no effect of already-low baseline RSA; instead of baseline autonomic activity, reduced autonomic flexibility is related to anxiety in adolescent girls from the general population. Our results suggest that in the general population, measures of autonomic reactivity are better predictors of future anxiety problems than measures of baseline autonomic activity. We should remark, however, that the effect size was small ($\Delta R^2 = 0.6\%$), and that the present study only assessed autonomic reactivity to a physical stressor. Although the orthostatic challenge test is a widely used standardised test to assess autonomic reactivity to a physical stressor, measures of autonomic reactivity to other stressors, such as mental challenge, might yield stronger effects.

As mentioned before, RSA has been generally accepted as an index for parasympathetic reactivity. Thus, we may conclude that low parasympathetic reactivity predicts future anxiety levels in girls. This is in line with

Table 3
Regression models with autonomic reactivity measures as predictors and anxiety 2 years later as the dependent.

Models/predictors		HR reactivity				RSA reactivity			
		B	*P*	ΔR^2	*F*(*df*)	*B*	*P*	ΔR^2	*F*(*df*)
Model 1	Main effect	0.001	0.373	0.1	0.79(1)	−0.004	0.640	<0.01	0.22(1)
Model 2	Main effect	0.001	0.187	7.7	38.5(2)	−0.008	0.280	7.7	38.2(2)
	Gender	−0.157	<0.01			−0.157	<0.01		
Model 3	Main effect	0.003	0.032	0.3	26.8(3)	−0.026	0.012	0.6	27.7(3)
	Gender	−0.156	<0.01			−0.156	<0.01		
	ANS*gender	−0.003	0.082			0.038	0.012		
Model 4	Main effect	0.003	0.058	0.3	20.8(4)	−0.026	0.013	0.4	21.8(4)
	Gender	−0.152	<0.01			−0.151	<0.01		
	ANS*gender	−0.003	0.103			0.038	0.011		
	Tanner stage	0.021	0.099			0.025	0.054		
Model 5	Main effect	0.002	0.078	19.5	70.5(5)	−0.021	0.022	19.4	71.3(5)
	Gender	−0.120	<0.01			−0.120	<0.01		
	ANS*gender	−0.003	0.073			0.032	0.018		
	Tanner stage	0.014	0.230			0.016	0.150		
	Anxiety wave 1	0.390	<0.01			0.389	<0.01		
Model 6	Main effect	0.002	0.083	0.1	58.9(6)	−0.020	0.024	0.1	59.5(6)
	Gender	−0.122	<0.01			−0.121	<0.01		
	ANS*gender	−0.003	0.071			0.032	0.016		
	Tanner stage	0.014	0.227			0.016	0.148		
	Anxiety wave 1	0.370	<0.01			0.368	<0.01		
	Depression wave 1	0.029	0.369			0.030	0.355		
Model 7	Main effect	0.000	0.906	28.5	168(7)	−0.007	0.336	28.3	168(7)
	Gender	−0.056	<0.01			−0.057	<0.01		
	ANS*gender	−0.001	0.458			0.011	0.309		
	Tanner stage	−0.003	0.755			−0.003	0.747		
	Anxiety wave 1	0.286	<0.01			0.286	<0.01		
	Depression wave 1	−0.163	<0.01			−0.164	<0.01		
	Depression wave 2	0.528	<0.01			0.526	<0.01		

HR = heart rate.
RSA = respiratory sinus arrhythmia.
Reactivity = difference between baseline measures in supine position and the measures in reaction to the orthostatic challenge test, active standing.
ANS = relevant autonomic measure, thus either HR reactivity or RSA reactivity.
B = beta; unstandardised regression coefficient.
P = probability, significance level.
ΔR^2 = percentage of *R* square change.
F(*df*) = *F*-value ANOVA (degrees of freedom).

the theory of Porges that relatively low parasympathetic reactivity may result in anxiety problems (Porges, 1995, 2001).

4.2. Gender differences

The present study revealed interesting gender differences in the relationship between autonomic flexibility and future anxiety levels. In girls, RSA reactivity predicted future anxiety levels, whereas no associations were found in boys. In our previous study (Greaves-Lord et al., 2007), we found that low baseline RSA was cross-sectionally associated with anxiety in boys, but not in girls. Thus, whereas low parasympathetic activity at rest was cross-sectionally associated with anxiety levels at age 10–13 in boys, low parasympathetic reactivity was prospectively associated with anxiety levels at age 13–15 in girls. Possibly, the association between RSA and anxiety changes across pubertal development. Both the incidence of anxiety problems and autonomic functioning are known to change across development, and to differ between sexes. Since the ANS and the gonodal system are known to interact (Stratakis and Chrousos, 1995), a certain level of sex-specific hormones may trigger reduced autonomic reactivity to additionally increase the risk for future anxiety problems. For instance, oestrogen is known to modulate autonomic function (Miller and Duckles, 2008), and increased testosterone levels have been associated with increased HRV (Wranicz et al., 2004). The fact that oestrogen and testosterone levels differ between boys and girls, and change across development, could underlie our mixed findings. In the present study, the effect of physical development was taken into account by adjusting for pubertal stage. However, levels of sex-specific hormones were not determined. Therefore, more research is needed to examine the specific effects of oestrogen, progesterone and testosterone on autonomic regulation and anxiety levels.

4.3. Specificity

The effect of RSA reactivity in girls disappeared when we adjusted for co-occurring depressive problems. This means that this association applies for internalising problems in general, and is not specific for anxiety.

While investigating autonomic flexibility in relation to anxiety, we chose to investigate anxiety as one general concept, instead of examining the separate anxiety disorders. This decision was made because biological variables (i.e., HR or RSA) are probably vulnerability markers for anxiety in general, whereas psychological variables (i.e., cognition) are probably vulnerability markers for the more specific anxiety disorders (Barlow, 2000). We should mention that in one previous study among adults, differences in autonomic activity were found between patients with panic attacks and patients with blood phobia (Friedman et al., 1993). However, in our general adolescent population sample, no evidence was found for distinct groups with their own specific anxiety symptoms (Ferdinand et al., 2006). Therefore, a more general approach of the concept of anxiety seems to be appropriate for the current study. Since the association between RSA reactivity and future anxiety in girls applies to internalising problems, and is not specific for anxiety, there is reason to believe that autonomic reactivity might in fact be a vulnerability marker for the broader domain of internalising problems.

4.4. Strengths, weaknesses and important issues

To our knowledge, the present study is the first to prospectively investigate the relationship between autonomic flexibility and anxiety in early adolescence. This study examined a large, representative sample of both boys and girls, which enabled us to investigate gender differences. Both HR and RSA were assessed, and confounders and co-occurring depressive problems were taken into account.

Of course, this study also has limitations. First, there was a lack of respiratory control in the assessment of the autonomic measures, whereas it has been argued that it might be important to use such procedures (Ritz and Dahme, 2006). Furthermore, sympathetic (re) activity was not determined. Some studies suggest that HRV in the low-frequency band mainly reflects sympathetic reactivity; however, preliminary analyses of our data indicated that HRV in the low-frequency band assessed in reaction to the orthostatic challenge task reflected sympathetic as well as parasympathetic reactivity (values dropped, while they would have increased if the measure was purely sympathetic); therefore, in this study it was not possible to report on sympathetic reactivity specifically. In future research, pre-ejection period or skin conduction should be determined, to gain more insight in sympathetic (re)activity.

The present study concerned individuals from the general population. Hence, anxiety levels were relatively low. This might, at least for some part, explain the small effect size that was found. Since participants were 13.6 years old on average at wave 2, and many anxiety symptoms such as social phobia and panic are known to increase after the age of 15 (e.g., Thyer et al., 1985; Wittchen et al., 1998), possibly associations between autonomic flexibility and anxiety become clearer later in life. Furthermore, it is known that anxiety emerges from a richly interconnected matrix of biopsychosocial variables (Friedman and Thayer, 1998b). Hence, autonomic flexibility should be regarded as one factor in a large group of joint risk factors for anxiety.

4.5. Clinical implications

In this general population sample, the association between measures of RSA reactivity and future anxiety levels was significant. Although this association was only found in girls, and the effect size was small, associations between RSA reactivity and anxiety were already observed in early adolescence. At this point, in the general population, RSA measures cannot be used for prevention purposes, that is, to identify individuals at risk for anxiety problems. Yet, our findings form a good basis for future studies in clinical populations. If future studies will point out that RSA reactivity plays a more important role in clinically diagnosed anxiety disorders – in the future – intervention programmes aimed at normalising autonomic functioning, such as relaxation training and slowed respiration, may be helpful (Sakakibara and Hayano, 1996). Such programmes might not only be effective for anxiety, but are probably also helpful in tackling depression.

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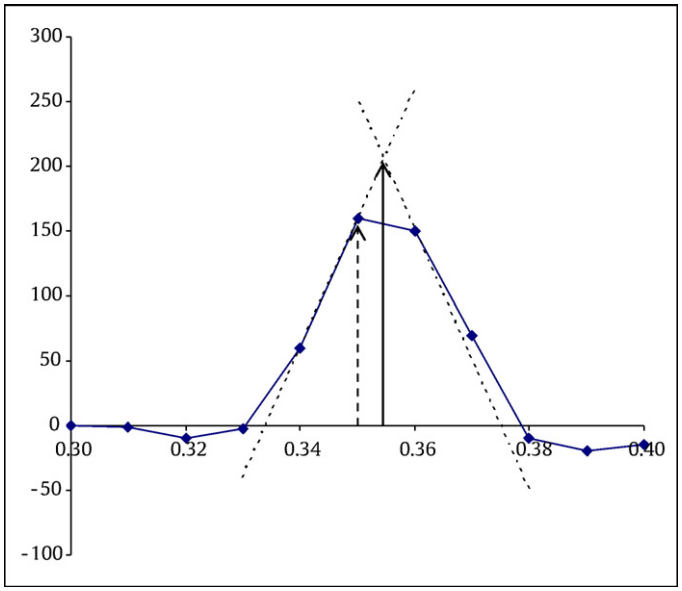
Appendix A. R-peak detection and interpolation algorithm

The detection of the *R*-peak is a simple maximum detection. However, at low sample rates (below 250 Hz), the true *R*-peak is usually not located close to the maximum but a few milliseconds before or after the maximum. To improve the time resolution, we have implemented an interpolation algorithm. It assumes that the *R*-peak is symmetrical near the true peak and that the steepest flank is representative for the flanks near the peak. In the figure, the method is shown. The dashed vertical arrow points to the *R*-peak detected as the simple maximum. The steepest flank is extrapolated to the next sample, and translated in the next sampled but now backwards to the maximum sample. The intersection of the two lines is the interpolated location of the true *R*-peak (solid arrow).



The improvement of the time resolution was determined for 100 Hz sampled data by comparing the interpolation results with 1000 Hz sampled data (simple maximum detection). It showed no more than ± 2 ms deviation. So, the time resolution improved from 10 to 4 ms and results in an approximated sample rate of 250 Hz.

Note that in normal cases the sample rate of 100 Hz is sufficient. In the table is shown what the contribution of the rounding error is for different sample rates. The lowest rate corresponds with generating a time series if HR was measured in bpm.

The contributions to the bands are calculated under the assumption that the rounding errors can be considered to be white noise and independent of the normal variability. Only at very low HRV levels, the 100 Hz precision is not sufficient (error IZ variance over 1% over the real variability). This can occur in cases of paced HR or severe

Fs [Hz]	Sample interval [ms]	Error variance [ms^2]	Contribution to LF [ms^2]	Contribution to HF [ms^2]
60	17	23.1	7.4	24.1
100	10	8.3	2.7	8.7
250	4	1.3	0.4	1.4
500	2	0.3	0.1	0.3
1000	1	0.1	0.0	0.1

autonomic failure. In our data set, mean LF and HF are over 1000 ms^2 and is the accuracy sufficient.

For more background information on this procedure, please see;

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