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Running head: STRESS REACTIVITY AND INCREASING ANXIETY

Impaired cardiac profile in adolescents with an increasing trajectory of anxiety when confronting an acute stressor

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Abstract

Maladaptive patterns of cardiac adjustment to stress in adolescents may reveal their vulnerability to anxiety disorders (ADs). Traditional research in this field has focused on anxiety levels, whereas the time course of anxiety has rarely been considered. Nevertheless, since overall anxiety decreases as adolescence progresses, increasing time courses are clinically relevant and can be associated with maladaptive contextual adjustment. In this study, the cardiac pattern of adjustment to stress in adolescents with increasing anxiety was analyzed. A sample of 44 adolescents (M = 14.88 years, SD = 0.53, 45.45% boys) were exposed to a socially relevant stress induction protocol, and their cardiac functioning was recorded. Participants with a trajectory of increasing anxious symptomatology over a twelve-month period (n = 24) showed attenuated heart rate levels in the stage of maximum stress in comparison to their non-increasing anxious counterparts ($p < .05$), as well as a heightened pattern of sample entropy throughout the stress induction ($p < .05$). These findings suggest a loss of cardiac flexibility in those adolescents at risk for ADs when confronting an acute stressor.

Keywords: Anxiety, trajectory, stress, heart rate variability, adolescence.

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Adolescence involves changes in physiological and emotional systems as young people move towards adulthood [1, 2]. Thus, it is not surprising that internalizing disorders are commonly suffered during this period [3]. Special attention must be paid to anxiety disorders (ADs), which have dramatic consequences on daily adolescent life and are strongly related to the development of severe mental disorders in adulthood [4, 5].

Adolescent anxiety may impact individuals' abilities to cope with daily events, especially those with a substantial emotional burden, such as stressful situations [6, 7]. One of the physiological systems that react more quickly and more contiguously to these conditions is the cardiovascular system; therefore, we can look at how it behaves under stress to see if there is any adjustment problem which could reveal an "at-risk" condition prior to the onset of an AD.

Adolescents have to cope with short-term stressors very commonly on a daily basis [8, 9]. Normative patterns of cardiac adjustment to stressors involve higher sympathetic dominance on the beating heart, measured as either higher heart rate (HR), spectral low-frequency band power, or scaling exponents, due to its energizing properties to face the demands of the stressor [10-12]. Once the confrontation is over, higher parasympathetic dominance (as shown by higher high-frequency band power of increased respiratory sinus arrhythmia) is expected to return to basal functioning. However, studies with AD adolescents have revealed a maladaptive pattern of adjustment, highlighting a blunted HR response when confronting a stressor and heightened respiratory sinus arrhythmia reactivity [7, 13]. Several authors have proposed a sympathetic withdrawal hypothesis to explain how AD patients confront a stressor, as adaptive physiological responses (i.e., the releasing of adrenal hormones) may not be triggered [6, 14]. Additionally, other studies have reported lowered cardiac flexibility in anxious patients, in terms of lower parasympathetic activation after confronting the anxiogenic or stressful stimulus among individuals suffering specific phobias or other anxiety disorders [15, 16]. Persistent maladaptive cardiac patterns to adjust to stressor have been linked with the development of cardiovascular disease and functional impairment throughout life [17, 18].

Cardiac adjustment to stressors has also been tested in subclinical samples, but the results are inconclusive. Some studies have highlighted the role of elevated levels of anxiety, or even anxious traits, as being responsible for impaired cardiac adjustment to stressors, but results have not been as consistent as expected, pointing to lower sympathetic activation when confrontation [19, 20]. Other studies have focused on the movement towards a full-blown diagnosis of an AD to suggest some impairment in adjusting to a stressor. Similarly, these results have been inconclusive [21-24].

Studying the risk for ADs in adolescence is particularly relevant due to its repercussions on mental health, because of its influence on the development of subsequent mental disorders, and due to its effects on maturation, for instance in personality and social skill development [4, 5, 25]. From the Research Domain Criteria (RDoC) framework (see [26, 27]) subclinical disorders or the so-called at-risk statuses are considered as falling along the same continuum than full-fledged disorders, qualitatively similar but quantitatively less severe [28, 29]. In this regard, longitudinal studies have demonstrated that significant increases in maladaptive anxiety, without considering specific syndromes, over a period of time could be a risk factor for ADs because: (1) maladaptive anxiety decreases in most individuals throughout adolescence [30, 31]; and (2) significant increases in anxious symptomatology (even without surpassing levels of clinical meaningfulness) may predict internalizing symptomatology, other negative outcomes over time, or the development of full-fledged syndromes [32-34]. Despite all of this, most studies ignore the trajectory of anxious symptomatology and rely only on the severity of anxious symptoms at one assessment time point.

Keeping in mind the RDoC principles, this study aimed to analyze the stress-induced cardiac profiles (arousal system domain, physiology level of analysis) of adolescents who exhibited an increasing trajectory of anxious symptomatology (negative valence system domain, self-reports level) along one year (and hence could be at risk for ADs), in the search for early biomarkers of anxiety. We hypothesized that at-risk participants would show a pattern of impaired flexibility when confronting a stressor. More concretely, we expected that adolescents with an increasing trajectory of anxious symptomatology, in comparison to participants with non-increasing trajectories, would show: (1) a lower responsiveness of the sympathetic branch of the autonomous nervous system when confronting a stressor in terms of lower HR and scaling exponents; (2) a lower withdrawal of the parasympathetic branch when a stressor was being confronted (i.e., higher high-frequency band power during the confrontation); and (3) higher

cardiac-related irregularity during a stressor confrontation and subsequent recovery (i.e., higher entropy levels). Since the trajectory of anxiety might be influenced by temperamental traits, we evaluated them to ensure that no differences existed between adolescents whose anxiety was increasing and those with non-increasing anxiety. Otherwise, cardiac changes under stress could be associated not only with the anxiety trajectory but with temperamental differences also.

Methods

Participants

An a priori sample size estimation indicated that 31 participants were needed to detect interaction effects within a multivariate analysis of variance design, assuming a medium effect size (f = .25), α = .05, 1 – β = .80, three predictors (an independent variable and two covariates) and two criteria with five levels of response. Calculations were carried out with G*Power 3.1.3 [35].

Eligible participants were Spanish adolescents with normative body mass indices who participated in the TrANS research project (see [36]). Participants were assessed for anxious symptomatology twice (once, and then again after twelve months). They agreed to participate by handing in a written consent form signed by themselves and their legal guardians. Participants who exhibited either a diagnosed AD or a severe cardiovascular disease were excluded. Likewise, participants who showed levels of anxious symptomatology lower than the 10th percentile in the RCADS total anxiety scale at the second assessment time were discarded. We initially assessed adolescents from 11 high schools to fulfil the sample size requirements (Figure 1). Finally, data from 44 participants (45.45% boys; M = 14.88 years, SD = 0.53) were gathered (Table 1).

The University Bioethics Committee approved the study procedures.

(Please, insert Figure 1)

(Please, insert Table 1)

Psychological assessment

The Early Adolescence Temperament Questionnaire (EATQ-Revised; [37]) is made up of 103 items with a 5-point Likert scale for assessing temperamental factors in accordance with Rothbart [38].

Two temperamental factors are considered as risk factors for anxiety disorders and are measured in this questionnaire: effortful control (EC) and negative affectivity (NA; an analogous construct related to the so-called fearful temperament [39]). These scales showed appropriate levels of reliability within our sample (Cronbach's α = .84 for EC, and α = .65 for NA).

The Revised Child Anxiety and Depression Scale (RCADS; [40]) is composed of 47 items with a 4-point response scale to evaluate the presence of ADs and depressive symptomatology. The RCADS was administered twice (once, and then again after twelve months). We only considered the total anxiety symptomatology scale, which showed adequate reliability at both measurement points (Cronbach's α = .94 at T1, and α = .97 at T2; correlation between points, $r = .70$, $p < .01$).

Diagnostic status.

The Mini-International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid; [41]) is a structured diagnostic interview based on DSM-IV and ICD-10 manuals. Due to the goals of this study, the AD modules of this interview were given (panic disorder, agoraphobia, separation anxiety disorder, social phobia, obsessive-compulsive disorder, posttraumatic stress disorder) by three trained postgraduate psychologists. Additionally, participants were asked if they suffered from any cardiac disease.

Cardiac measures

The mean heart rate (HR), the square root of the mean of the squares of the successive differences between adjacent NN peaks (rMSSD), in log-linear scale; and the high-frequency band (HF; 0.15–0.40 Hz) spectral power (both also in log-linear scale) were calculated [42]. HF is related to parasympathetic activity in cardiac signals [21].

Detrended fluctuation analysis.

Detrended fluctuation analysis (DFA) allows for the estimation of temporal correlations of power-law form embedded in interbeat interval (IBI) time series [43]. DFA calculations involved that the IBIs time series is integrated and divided into boxes of equal length, *n*. Afterwards, a least-squares line is fit to the data, representing the trend in each box. The root-mean-square fluctuation of this integrated and detrended time series is calculated for each box by (see Equation 1)

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[y(k) - y_n(k)]^2} \qquad \text{[Equation 1]}$$

This computation provides a relationship between $F(n)$ and the box size n. Typically, $F(n)$ increases with box size n. A linear relationship on a log-log graph indicates the presence of scaling, and the slope of the line is the scaling exponent α.

Two scaling exponents can be calculated: the short-term $\alpha 1$ exponents (4-11 heart beats), and the long-term $\alpha 2$ exponents (> 11 beats). We only used the $\alpha 1$ exponents due to the length of the study stages. Exponents with values around 0.50 signify uncorrelated data; values from 0.50-1 reflect long-range correlations, and values around 1.50 indicate stochastic data (Brownian noise).

Sample entropy.

Sample entropy (SampEn) is a measure of chaotic irregularity or complexity inherent to a biological system over time [44]. SampEn is the negative logarithm of the probability that if two sets of data points of length m have distance < r then two sets of simultaneous data points of length m+1 also have distance < r (see Equation 2). Embedding dimension (length m) was set to 2, and tolerance r was set to 20% of the standard deviation of the data in the corresponding time series.

$$\text{SampEn}(m, r, N) = -\ln[C^m(r)/C^{m+1}(r)] \qquad \text{[Equation 2]}$$

Procedure

Potential participants filled out the RCADS (T1) and twelve months later the EATQ-R and the RCADS again (T2). Afterwards, the diagnostic interview was administered.

Once recruited and after the T2 assessment, participants underwent the stress induction protocol based on the Trier Social Stress Test for Groups (TSST-G; [45]). This protocol induces moderate to high levels of stress-related responses by means of a social-evaluative stimulus considered threatening, challenging, and unexpected [46].

Participants from the same high school were gathered in groups (M = 3.86 individuals, SD = 1.87, range from 3 to 8) on a regular school day, and the protocol depicted in Figure 2 was applied. We ensured that participants did not take any caffeinated or alcoholic drink or were not menstruating before experimental session started. Cardiac activity was recorded for 3 minutes under resting conditions (participant should be siiting and relaxed). Afterwards, a researcher explained the task to be performed:

participants had to stand up and present themselves, individually. The presentation was one-minute long, in front of a video camera, and with two researchers and the group members as an audience (exposition stage). Beforehand, participants were given a 3-minute period in which to think about their performance (anticipation stage), and right after that period, a researcher informed them about the presentation order, which was decided upon at random. Then, the first participant started presenting. Researchers provoked participants when they became silent while presenting (e.g., by saying "You still have some time left. Please continue!"). After the one-minute presentation, participants went back to their seats, and the following three minutes were recorded and considered as the recovery stage.

(insert Figure 2 here)

Data acquisition and preprocessing

IBI time series were recorded continuously at 1000 Hz, using the Firstbeat Bodyguard 2© (Firstbeat Technologies Ltd., Jyväskylä, Finland). This device was put on the skin with two electrodes: one on the left side of the chest and the other under the collar bone on the right side of the chest. The whole recording was segmented into consecutive non-overlapping time series: baseline (3 minutes), anticipation stage (3 minutes), pre-exposition (1 minute), exposition (1 minute), and recovery (3 minutes). The pre-exposition stage (i.e., the minute before starting the presentation) was used to control for order effects.

The IBI time series were filtered with the Physionet HRV analysis toolkit (see https://physionet.org/tutorials/hrv-toolkit/#basic-time-and-frequency-domain-measures): a low-pass band filter at 1100 ms, a high-pass band filter at 400 ms, and a central interval filter were applied. As a result of filtering, 9250 IBIs were excluded (out of 106210, i.e., 8.23%). Cardiac measures were calculated from the filtered IBI series using Kubios HRV 2.1 [47].

Analytic strategy

To test the study hypotheses, analysis of variance (ANOVA) was used, and the different study stages were considered as repeated measures. In line with Young and Benton [48], the cardiac measures were grouped and two multivariate ANOVAs (MANOVAs) were performed: (1) time-domain MANOVA (HR and rMSSD), and (2) frequency-domain MANOVA (HF and DFA $\alpha 1$). Additionally, a univariate

ANOVA was conducted for SampEn. The trajectory of anxiety was used as a between-group factor. The total anxiety score in the RCADS at T2 was subtracted from the score at T1, and participants with positive values (with higher anxiety symptomatology at T2 in comparison to T1, therefore) took part of the increasing anxiety (IA) group. The remaining participants were put in the non-increasing anxiety (NIA) group. This grouping strategy mirrors the classification of adolescents in the at-risk and not-at-risk categories, since increased anxiety symptomatology has been seen as a risk factor for AD.

Cardiac measures (except for HR) were weighted by the inverse of the baseline HR to control for HR-derived mathematical bias [49, 50]. Moreover, gender was introduced as covariate, following scientific literature suggestions (see, for instance, [10, 13]). According to Senn [51], other covariates should be added if: (1) significant between-group differences were found for any psychological variable, (2) a linear association was described between that variable and the criterion, and (3) no independent variable*covariate interaction was observed.

Bonferroni post hoc tests were used to detect within-stage differences and for between-group differences within each stage. The Greenhouse-Geisser correction was applied when the assumption of between-group variance homogeneity was violated.

IBM SPSS v. 20 was used for analyses.

Results

No differences in temperamental traits were found. On the other hand, participants with increasing trajectory had significantly higher levels of anxious symptomatology at T2 (see Table 1). We therefore incorporated the symptomatology at T2 as a covariate.

The time-domain MANOVA revealed a significant stage effect for HR, F (4, 160) = 9.80, p < .01, $\eta^2_{partial}$ = .20; and rMSSD, F (4, 160) = 5.01, p < .01, $\eta^2_{partial}$ = .11. Thus, an increase in HR and a decrease in rMSSD over induction stages were shown, independent of the study groups, as well as opposite patterns when considering the recovery stage (Table 2). Additionally, an interaction HR*group was found with, F (4, 160) = 2.62, p < .05, $\eta^2_{partial}$ = .06, pointing to lower HR in the exposition stage in participants in the IA group. Post hoc between-group differences confirmed these lower levels in the exposition stage, t (42) = 2.42, p < .05. No other main effects nor interaction or covariate effects were found on these dependent variables.

(Please, insert Table 2)

The frequency-domain MANOVA revealed a significant main effect of stage on HF, F (2.95, 115.16) = 3.56, $p < .05$, $\eta^2_{partial} = .08$; and the DFA exponent, F (4, 156) = 7.12, $p < .01$, $\eta^2_{partial} = .15$. These results indicate that the scaling exponent increased throughout the stages and bounced back during recovery, and an opposite pattern was revealed for the HF. A stage*gender interaction effect was observed for the HF power, F (2.95, 115.16) = 3.07, $p < .01$, $\eta^2_{partial} = .07$. In turn, lower levels of HF power were shown for girls in the exposition stage. On the other hand, an stage*anxiety symptomatology at T2 was observed, with F (4, 156) = 2.70, $p < .05$, $\eta^2_{partial} = .06$, pointing to lower DFA exponent in participants with higher anxiety at T2. No other significant effects were found for these dependent variables.

Finally, regarding SampEn, a main effect of stage was found, F (4, 156) = 8.18, $p < .01$, $\eta^2_{partial} = .17$; and a between-group effect was revealed, F (1, 39) = 4.29, $p < .05$, $\eta^2_{partial} = .10$, signaling higher levels of entropy in IA participants across the study stages (Figure 3). Post-hoc between-group differences were revealed for the anticipation stage, t (42) = -2.31, $p < .05$; being marginally significant for the exposition stage, t (42) = -1.80, $p = .080$. No other significant post-hoc differences were found.

(Please, insert Figure 3)

Discussion

Anxiety disorders entail significant degree of mental distress and important sanitary cost. It is highly revelant to find biomarkers that help clinicians and researchers to identify individuals with a clear risk of suffering an AD to plan early interventions to hinder symptom perpetuation. This study examined the cardiac profiles of adolescents with increasing anxious symptomatology over a twelve-month period (as an at-risk factor for AD) when confronting an acute stressor. Cardiac measures were selected to evaluate the influence of the sympathetic and parasympathetic branches of the autonomous nervous system, as well as their interplay on the heartbeat.

Normative patterns of adjustment to stress involve physiological (cardiac) changes to facilitate effective emotional regulation [52, 53]. Thus, a sympathetic dominance (increased heart rate) should

promote physiological arousal in order to cope with the stressor. However, at-risk participants exhibited a pattern of attenuated heart rate, significantly marked in the exposition stage. The expected sympathetic activation reflects the flexibility of the cardiac system to adapt to the stressor, and therefore at-risk participants would be expected to show a loss of cardiac flexibility when confronting an acute stressor.

Some studies relying on high levels of anxious symptomatology as subthreshold conditions to full diagnoses of ADs have shown relatively similar blunted HRs. In one of these studies, Schmitz et al. [20] observed lower HR levels in young high-social anxious adolescents when confronting a stressor. Nevertheless, other studies have found a lack of influence of anxious symptomatology on HR profiles when a stressor is confronted [19, 54]. Since these studies did not take the time course of anxiety into account, the role of the trajectory of anxiety on the reported results could not be evaluated, but based on the results of this study, we suggest that the course of anxiety over time does, in fact, alter cardiac adjustment to acute stressors in addition to (or more than) the level of symptomatology at a specific point in time. The absence of a significant effect of the anxiety levels at T2 in terms of the majority of the cardiac measures studied, as we found, also supports this finding. Therefore, an early biomarker of anxiety in a subclinical, at-risk status, would be the HR hypoactivity when confronting the stressor.

The anxiety symptomatology at T2 did play a role on the DFA exponent according to our results. In general terms, the DFA exponent tends to become higher when individuals are exposed to a stressor [11, 12]. Our results endorsed that finding. However, we observed that participants who showed higher levels of anxiety at T2 exhibited a lower exponent, as a reflection of a worse cardiac adjustment to stressor demands (see [55]).

On the other hand, significantly different between-group profiles of HF power were not found when undergoing the stress induction protocol. In other words, an increasing trajectory of anxious symptomatology was not associated with lower parasympathetic withdrawal under stressful conditions. Studies with subclinical anxious samples have also shown results along this same line [20]. This may suggest that maladaptive patterns of parasympathetic dominance occur when an AD is fully expressed but not when anxiety remains at a subclinical level. In fact, parasympathetic dysregulation is a key marker of ADs in tonic (baseline conditions), as well as in phasic (reactivity tasks) conditions [7, 53, 56].

Finally, at-risk participants showed a pattern of heightened sample entropy when confronting the stressor (which was significantly marked in the anticipation and marginally significant in the exposition

stage). This pattern, like the pattern of sympathetic under activation, seems opposite to the normative pattern of decreases in stress-induced entropy reported in other studies [11,57, 58]. Hence, the loss of flexibility reflected by the attenuated HR response can be seen again in the less-reduced entropy pattern shown by at-risk adolescents. Because entropy in heart rate signals has been linked with a gradual loss of sympathetic dominance and lower vagal withdrawal in head-up tilt tests [59, 60], this pattern suggests an impaired sympathetic-parasympathetic interplay when confronting a stressor.

To sum up, this study reveals that the trajectory of anxiety symptomatology should be taken into account when researching the patterns of adjustment to stress in adolescents. In this regard, and from a more domain-related approach, the loss of cardiac flexibility turned out to be a biomarker of anxiety symptom escalation (unlike effects of a drastic increase of anxiety symptoms as a consequence of suffering a traumatic event, for instance, being raped or witnessing a severe accident) when confronting stressors, due to a lower sympathetic dominance (shown by lower HR and higher SampEn). Moreover, it demonstrated that stress induction protocols may be a very useful protocols to examine how anxiety symptomatology may affect the cardiac system in a crucial period of maturation, as adolescence is.

The main strength of our study is that it is based on an experimental task with an important socially-relevant component. Stressful social situations are very common in adolescence, and therefore, this specific task enhances the ecological validity of the results. Moreover, several controls to improve the explanatory power of our findings were added (for instance, the order of presenting or the mathematical control of the HR-derived drift on cardiac measures).

One of the shortcomings of the study is the relatively low sample size that could threaten the generalizability of our findings. An a priori sample size estimation affirmed the adequacy of our sample, but further studies with larger samples would be helpful to corroborate the findings reported here. The severity of anxiety should be addressed also in future studies. The IA group in this study could include adolescents whose anxiety scores went from 20 at T1 to 25 at T2, but also participants showing scores as low as 11 at T1 and 15 at T2. Although, this is not contradictory with the aims of the study, admittedly the absolute levels of anxiety should be taken into account on further research studies. A second shortcoming is that we did not use measures related to other psychophysiological systems (i.e., endocrine system or self-report). We focused on heart-derived functioning because of its measurability, its feasibility to be studied in other contexts (hospital settings, laboratory, etc.), and its sensitivity to

changing conditions. Equipment needed to study heart's functioning, even under ecological conditions, is relatively affordable (see, for instance [61]). Additionally, some evidence supports the significant relevance of cardiac measures as markers for mental disorders and maladaptive functioning [21, 62]. However, physiological measures other than cardiac ones would help to depict an overall pattern of adjustment to stress. An additional issue is the relatively short period of baseline recordings. However, and according to Camm et al. [42], a minimum of 1 minute is needed to assess the frequency-domain measures of power. Further research should be done with larger periods of recording to support conclusions derived from this study. Finally, more concern should have been given to the role of temperamental traits as other risk factors for ADs in this study. However, effortful control and negative affectivity, two fundamental traits that have been repeatedly associated with ADs, were controlled for in this study. Further research should be designed to elucidate more clearly if (and how) these traits could be associated with the time course of anxiety throughout adolescence.

Adolescence is a turbulent period in life. Consequences derived from a maladaptive response to daily conditions may become serious in terms of wellbeing and health as adolescents move towards adulthood. This study provides some valuable evidence following the RDoC approach which allows studying the complex phenomenon of anxiety from multiple, complementary levels of analysis (concretely, we studied the negative valence system domain through anxiety symptomatology at a self-report level; and the arousal system domain through examining the cardiac reactivity to acute stressors at a physiology level of analysis). Moreover, the study sheds light on how an increasing course of anxious symptomatology in adolescents may lead to a maladaptive adjustment to acute stressors, which come up very frequently throughout this period. The loss of cardiac flexibility when confronting stressors may be used as a preventive measure to identify individuals at risk for anxiety disorders. The next straightforward step would be to make psychological interventions to reduce the impact of anxiety on a daily basis and encourage adolescents to confront stressful conditions in their daily life in a more adaptive way. Potential successful preventive programs could be the transdiagnostic treatments (see, for instance, [63, 64]) focused on core psychopathology or distressing symptoms that impede a successful contextual adjustment. On the other hand, HRV biofeedback [65] can be a useful treatment focused on the impaired cardiac flexibility shown by adolescents with increasing anxiety symptoms.

Funding and conflict of interest

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The authors of this manuscript declare that they have no conflict of interest.

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Table 1

Descriptive features for the study groups

	NIA group (n = 20)	IA group (n = 24)	χ^2/t^*	*df*	*p*	Effect size†
Gender (% boys)	30	41.67	0.64	1	0.42	.12
Age	14.92 (0.57)	14.85 (0.48)	0.42	38	.68	.13
High school area (% urban)	40	33.33	0.21	1	.65	.07
Temperament						
EC	4.37 (0.50)	4.11 (0.39)	1.92	42	.06	0.58
NA	2.85 (0.61)	3.13 (0.50)	-1.66	42	.07	-0.50
Anxious symptomatology						
T1	32.20 (19.22)	28.21 (15.88)	0.76	42	.45	0.23
T2	21.90 (15.78)	42.54 (20.45)	-3.69	42	< .01	-1.13
Change T2-T1‡	-10.33 (9.82)	14.33 (11.40)	-7.59	42	< .01	-2.32

Note. Mean and standard deviations (between brackets) displayed for numerical data, and percentage of cases for categorical data.

NIA group = Non-increasing anxiety group; IA group = Increasing anxiety group; EC = Effortful control; NA = Negative affectivity.

T1 refers to the first measurement point. The T2 measurement point was done 12 months after T1.

* χ^2 tests for categorical data and *t* tests for numerical variables.

† Cramer's ϕ for categorical data and Cohen's *d* for numerical data.

‡ Difference between measurement point in anxious symptomatology.

Table 2

Cardiac measures across the study stages

		Stage				
		Baseline	Anticipation	Preexposition	Exposition	Recovery
HR						
	NIA group	83.60 (12.81)	88.80 (11.37)	96.89 (15.85)	109.11 (15.76)	84.92 (13.33)
	IA group	80.35 (9.61)	86.94 (11.23)	93.19 (14.78)	101.74 (15.56)	84.23 (11.55)
rMSSD						
	NIA group	3.72 (0.50)	3.58 (0.42)	3.40 (0.52)	3.10 (0.57)	3.68 (0.47)
	IA group	3.95 (0.39)	3.72 (0.40)	3.62 (0.56)	3.38 (0.56)	3.85 (0.43)
HF						
	NIA group	6.56 (1.07)	6.15 (0.88)	5.92 (1.05)	5.28 (1.21)	6.38 (0.86)
	IA group	7.15 (0.79)	6.63 (0.69)	6.40 (1.08)	6.02 (1.04)	6.82 (0.96)
DFA α1						
	NIA group	1.20 (0.19)	1.30 (0.16)	1.41 (0.21)	1.52 (0.24)	1.26 (0.20)
	IA group	1.10 (0.27)	1.27 (0.24)	1.34 (0.20)	1.35 (0.23)	1.21 (0.22)
SampEn						
	NIA group	1.25 (0.35)	1.20 (0.32)	0.91 (0.38)	0.66 (0.29)	1.20 (0.36)
	IA group	1.43 (0.34)	1.31 (0.37)	0.97 (0.45)	0.97 (0.45)	1.24 (0.38)

Note. Measures are displayed as means and standard deviations (between brackets).

A main stage effect was found for every cardiac measure ($p < .05$). Pairwise comparisons revealed differences between stages ($p < .05$, for all comparisons), but not between the baseline and recovery, nor between the anticipation and preexposition.

HR = Heart rate (bpm); rMSSD = Square root of the mean of the sum of the squares of differences between adjacent interbeat intervals (ms) in log lineal scale; HF = High-frequency band power (ms^2) in loglineal scale; DFA α1 = Short-term scaling exponents; SampEn = Sample entropy. NIA groups = Non-increasing anxiety group; IA group = Increasing anxiety group.

Figure 1. Flow diagram of sample selection.

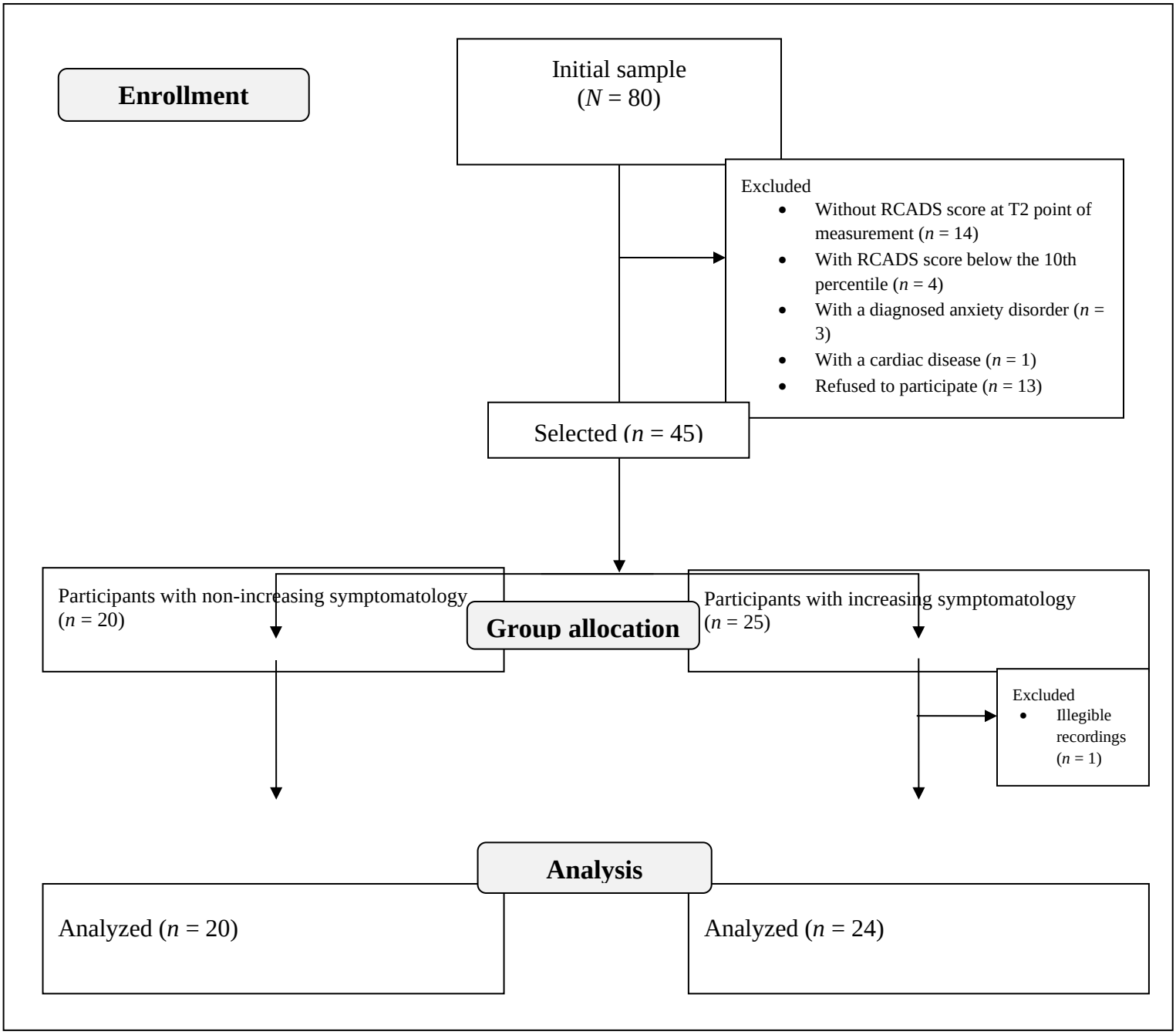


Figure 2. Summary of the study stages.

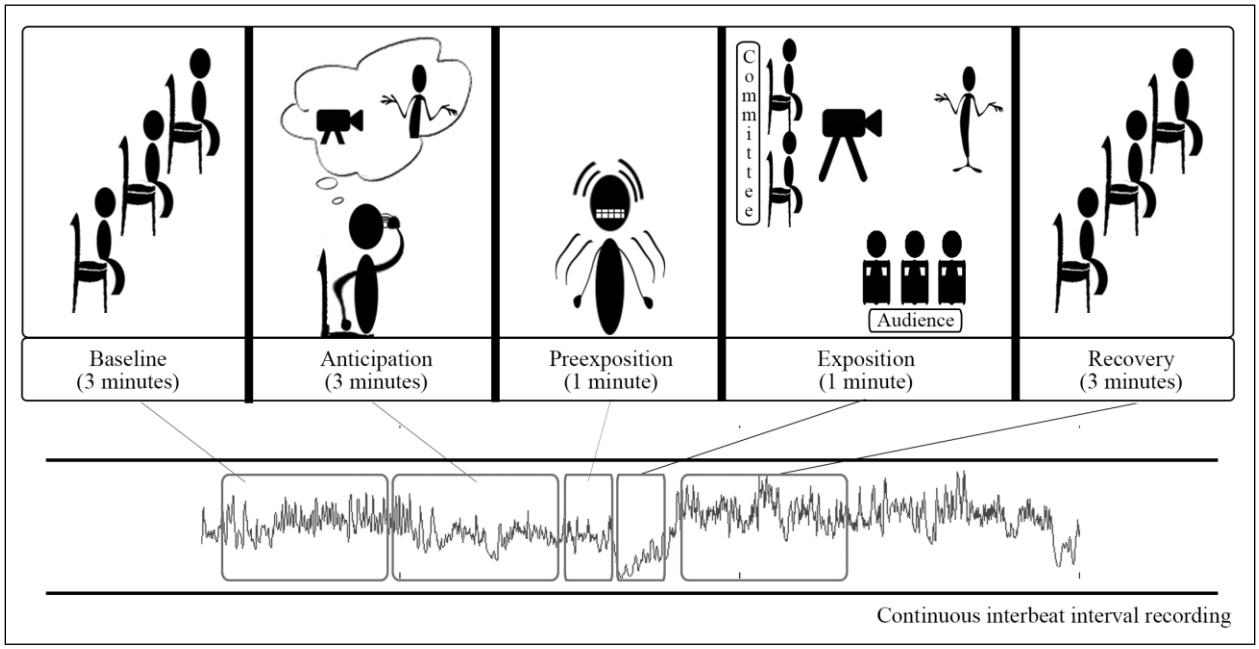
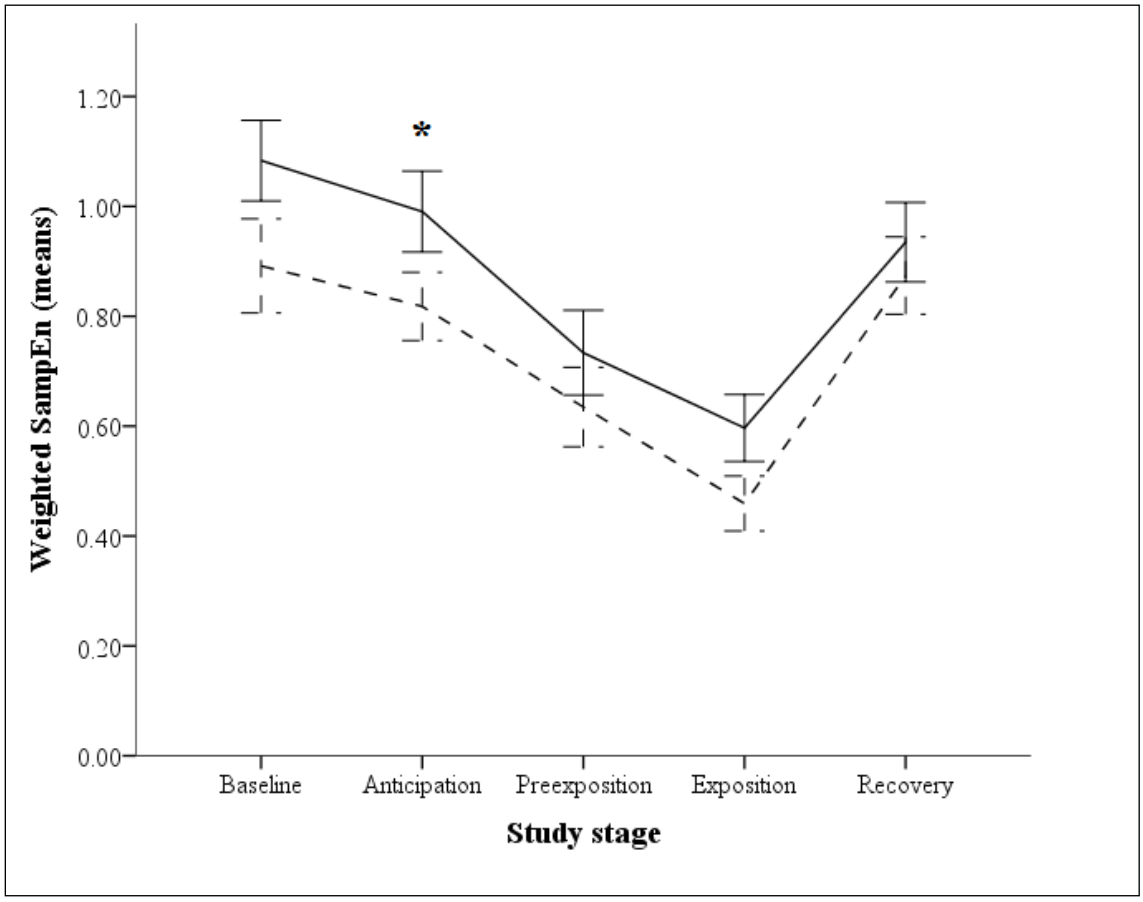


Figure 3. Weighted sample entropy across the study stages.



Note. Error bars depict the mean standard error.

Dashed line = Non-increasing group; solid line = Increasing anxiety group.

SampEn = Sample entropy (weighted by the mean heart rate at baseline stage).

* Between-group differences within this stage ($p < .05$).