Universitat
de les Illes Balears

DOCTORAL THESIS
– 2017 –

COMPLEX TRAJECTORIES OF ANXIETY ACROSS ADOLESCENCE

TOWARDS THE DISCOVERY OF NEW MARKERS FOR EARLY DETECTION AND TREATMENT

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Doctoral Program of Neuroscience

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ALEJANDRO DE LA TORRE LUQUE

Director: Dr. Xavier Bornas i Agusti
Tutor: Dr. Xavier Bornas i Agusti

Doctor by the Universitat de les Illes Balears

"The pursuit of PhD is an enduring daring adventure."

Lailah Gifty Akita

"In the depth of winter, I finally learned that within me there lay an invincible summer."

Albert Camus

"You can't cross the sea merely by standing and staring at the water."

Rabindranath Tagore

Dr. Xavier Bornas i Agusti, of the University of the Balearic Islands

I DECLARE:

That the thesis '*Complex Trajectories of Anxiety across Adolescence: Towards the Discovery of New Markers for Early Detection and Treatment*', presented by Mr. Alejandro de la Torre Luque to obtain a doctoral degree, has been completed under my supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, I hereby sign this document.

Palma, Spain

Date: 27 July 2017

This doctoral dissertation constitutes an article-based thesis opting for an international doctorate. The thesis is composed of five journal articles:

De la Torre-Luque, A., Bornas, X., Balle, M., & Fiol-Veny, A. (2016). Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. *Neuroscience & Biobehavioral Reviews, 68*, 410-422. doi: 10.1016/j.neubiorev.2016.05.023 (JCR® Impact Factor = 8.30)

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SYMBOLS AND ABBREVIATIONS

5-HT	Serotonin
AC	Attentional Control
AD	Anxiety Disorder
ANS	Autonomous Nervous System
APA	American Psychological Association
AS	Anxiety Sensitivity
BIS	Behavioral Inhibition System
CARACAW	Center for Applied Research and Assessment in Child and Adolescent Well-being
CO_2	Carbon Dioxide
DA	Dopamine
DST	Dynamical Systems Theory
EC	Effortful Control
ECG	Electrocardiagraphy
EEG	Electroencephalography
EMA	Ecological Momentary Assessment
EP	Evoked Potential
ERP	Event-related Potential
FT	Fearful Temperament
GAD	Generalized Anxiety Disorder
HPA	Hypothalamic-pituitary-adrenal Axis
HR	Heart Rate
HRV	Heart Rate Variability
Hz	Herz (unit of frequency)
ITC	International Test Commission
MEG	Magnetoencephalography
NA	Negative Affectivity
NE	Norepinephrine
NN	Nornal-to-normal interval of heartbeat
OCD	Obssessive Compulsive Disorder

PNS	Peripheral Nervous System
PSG	Polysomnography
PTSD	Posttraumatic Stress Disorder
RMSSD	Square Root of the Mean of the Sum of the Squares of Differences between Adjacent NN Intervals
SDNN	Standard Deviation of all NN intervals
SSL	Super Skills for Life Program
WHO	World Health Organization

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PREFACE

This doctoral dissertation has been conceived and developed within the framework of a research project funding by the Spanish Ministry of Economy and Competitiveness, and entitled "Complex trajectories of anxiety in adolescents: towards a better prediction of the onset of anxiety disorders" (project ref.: PSI2012-34780).

This research project was drawn out and was being carried out at the same time that some events affecting the education system and some structural concerns on mental health were ongoing. These factors determined meaningfully the directions, decisions and approaches followed in the current dissertation. Firstly, some groundbreaking clinical and scientific findings in mental health were obtained, putting into question how psychopathology was addressed by clinicians and researchers thus far. As a result, an update on the reference diagnostic manuals was conducted. In 2013, the American Psychiatric Association released the fifth version of its Diagnostic and Statistical Manual of Mental Disorders. Substantial refinements were incorporated into this manual in comparison to the previous one. Nevertheless, many theorists kept criticizing that traditional and rigid approach to conceptualize the sources of mental distress as categorical, opaque entities. A more flexible way to account for the continuum from normal behavior to disease was needed. In 2009, the National Institute of Mental Health launched the Research Domain Criteria (RDoC) Initiative. This project was created as more focused on a research basis, and was aimed at clarifying mental disorders taking into consideration different levels of analysis (physiological, self-reported, etc.), but not categories neither diagnostic entities. Many researchers are agreed that a better conceptualization is derived from the RDoC. Additionally, constructs typically considered in developmental psychopathology such as developmental trajectories and sensitive periods of development are well addressed from this perspective. Therefore, a research-domain perspective was adopted in the current doctoral dissertation. This means that varying sources of psychological distress (different points along the continuum of health and psychopathology) were considered in the studies composing the dissertation.

On the other hand, the autonomous government of the Balearic Islands released a very controversial education law just when the studies composing this dissertation started carrying out (end of 2013 and beginning of 2014). As a consequence, many student and teaching staff strikes in secondary schools were done for some months hindering and delaying the study schedule. However, every cloud has a silver lining and learning gained from these circumstances is highly valuable. Different strategies to address difficulties and new tools to solve problematic situations have been obtained as the research questions considered in this thesis dissertation were responded.

Finally, it is noteworthy how we addressed the study aims of this research project. From an eminently clinical framework, I can say that I have obtained some learning on how anxiety evolves across adolescence from capturing moments and interactions with adolescents, legal guards or parents and secondary school teachers. I am very proud of how much I have discovered from the integrative perspective adopted to analyze the complex nature of anxious phenomenon. I am totally sure that all of moments lived across the project course and dissertation writing out have constituted actual events promoting growth as a person.

Once these considerations have been mentioned, the current doctoral dissertation aimed at responding to some research questions, in keeping with a psychopathological and developmental perspective, and integrating some evidence from ecological and controlled settings. The key questions to respond were: *might some subjective and/or objective markers enable the characterization of different conditions on the continuum of maladaptive anxiety in adolescence*? Finally, it aimed to respond to: *how might a psychological preventive intervention revolve trajectories of anxiety which put individuals at risk for developing anxiety disorders?*

The current thesis gathers some findings from five studies to respond to these research questions. The first study constituted a meta-analysis to identify relevant measures from a nonlinear perspective in the search for biomarkers of anxiety disorders (as well as for other emotional disorders). The second study was conducted from a longitudinal perspective to analyze how anxiety evolves from early to middle adolescence. In this case, it intended to depict the varying dynamic trajectories of anxiety symptomatology from age 12-13 to age 15-16 and how some risk factors (gender and temperamental factors) might determine at-risk trajectories. The third and

fourth studies aimed to characterize how the cardiac system of adolescents at risk for anxiety disorders worked in ecological conditions (third study) and undergoing a controlled paradigm of stress induction (fourth study). In this regard, different conceptualizations of at-risk individuals (high levels of anxious symptomatology, cross-sectionally; or an increasing trajectory of symptomatology, longitudinally) were followed across studies. Finally, the fifth study was performed to assess the efficacy of a preventive program to revolve the increasing trajectory of anxious symptomatology in adolescents, by means of a transdiagnostic program to tackle internalizing symptomatology.

Relevant findings were extracted from these studies. They may constitute an important contribution to a wider understanding of anxiety disorders and how crucial they are in adolescence. Moreover, useful tools are described in this doctoral dissertation in order to accurately assess and ameliorate anxious symptomatology and to promote a higher sense of wellbeing among adolescents, a period in life unique with many challenges and opportunities which deserve to be lived.

I invite clinicians and researchers of human sciences to immerse themselves into this thesis dissertation in order to explore new ways to address the complex phenomenon of anxiety.

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Alejandro de la Torre.

Palma, 19 June 2017.

CONTENTS

Thesis Summary 0

ABSTRACT

Anxiety constitutes an actual health hazard for contemporary societies with a dramatic impact on economy and daily life. Current diagnostic manuals take a look at anxiety just considering the full-blown anxiety disorders (ADs). Unfortunately, individuals who do not fully match all the AD criteria, but suffer significantly from anxiety symptomatology, have systematically been overlooked for mental health care. For that reason, a more flexible, non-categorical approach to deal with psychopathology is needed to be followed. The Research Domain of Criteria (RDoC) initiative proposes a multidimensional (neither static nor categorical) standpoint for anxiety to be studied. Thus, varying sources of distress, either clinical or subclinical, would be considered from this framework. This is especially relevant in the study of adolescent anxiety. Anxiety in adolescence is proved to be a strong predictor of different mental disorders in adulthood (e.g., depression, substance abuse disorders, etc.), even when anxiety symptomatology does not reach the level of clinical meaningfulness. Therefore, it turns into crucial to identify sensitive markers of adolescent anxiety which guide clinical assessment and intervention. In this regard, recent lines of research have highlighted how valuable are the measures derived from the Dynamical Systems Theory (DST) in featuring human socioemotional and physiological systems of individuals with psychological problems (identifying biomarkers, clarifying mechanisms of action, etc.). The main goal of this doctoral dissertation was to shed light on the search for accurate markers of anxiety in adolescence, allowing mapping individuals experiencing varying conditions onto the continuum of anxiety. To satisfy this aim, a meta-analytic study of scientific literature review (Study 1) and three empirical studies were conducted. The first empirical study (Study 2 within the thesis) covered the subjective research domain (by means of self-reports), and the two others (Study 3 and 4) were focused on identifying markers in the physiological research domain (in the cardiac system, concretely). As a secondary aim, it was intended to provide and test a prevention program to ameliorate the symptomatology of individuals suffering an anxiety-related condition. This aim was satisfied by performing a study (Study 5) to test the efficacy of

an evidence-based preventive program for ameliorating internalizing symptomatology in adolescence. To summarize the relevant findings from the five studies mentioned, some relevant measures (e.g., sample entropy or fractal dimension in the cardiac, brain and hormonal systems) turned out to be sensitive for ADs were found from the Study 1. Regarding the Study 2, a depiction of the trajectory of anxiety symptomatology from early to middle adolescence was provided, highlighting the dynamical influence of some risk factors (temperament and gender) on its developmental course. The Study 3 and 4 enabled the identification of some cardiac biomarkers in adolescents with several conditions of subclinical anxiety: the allometric exponent *h* was proved to be an accurate biomarker in ecological contexts (Study 3); moreover, the sample entropy index turned out to be valuable when adolescents underwent a laboratory-based stress-induction task (Study 4). Finally, the Study 5 demonstrated that the preventive program delivered cut out efficiently the rising trajectory of anxiety observed in some adolescents as a risk factor for full-blown ADs. To sum up, some relevant markers (subjective and cardiac-derived) were identified from the studies conducted within this doctoral dissertation to map individuals onto the anxiety continuum. These markers were found as a result of following a flexible, developmental and psychopathological perspective in the study of anxiety in adolescence. Relevant implications for clinical assessment and intervention are derived from this doctoral dissertation in favor of planning tailored-based protocols and the promotion of prevention policies in order to facilitate healthier socioemotional maturation in adolescence.

RESUMEN

La ansiedad es uno de los principales problemas de salud pública en las sociedades actuales dado su impacto económico y en la vida diaria. Los manuales diagnósticos dan cuenta de esta problemática a través de los llamados trastornos de ansiedad (TA). Desafortunadamente, muchas condiciones ansiosas con alta afectación en el bienestar personal no se encuadran dentro del diagnóstico de estos trastornos y, por ende, no son objeto de tratamiento. Por ello, se hace preciso incorporar sistemas de abordaje más flexibles y no categoriales. En este sentido, cabe destacar la iniciativa de los Criterios del Dominio de Investigación (CDI) por su enfoque multidimensional (no estático ni categorial) en el estudio de la ansiedad. Este enfoque cubriría condiciones clínicas y subclínicas que causan malestar significativo. La iniciativa CDI es especialmente indicada para el estudio de la ansiedad en la adolescencia, principalmente porque los problemas de ansiedad en este periodo vital, incluso en niveles subclínicos, son predictores significativos del desarrollo de diversos problemas mentales en la adultez (trastornos depresivos, trastorno de abuso de sustancias, etc.). Por ello, se hace crucial adoptar una perspectiva multidimensional en la búsqueda de marcadores de la ansiedad en la adolescencia que guíen la práctica clínica. La Teoría de los sistemas dinámicos (TSD) puede contribuir significativamente a este propósito, dadas las evidencias desde esta perspectiva en la detección de marcadores socioemocionales y fisiológicos en individuos con diversos problemas psicológicos. En esta tesis doctoral se pretendía contribuir en la búsqueda de marcadores de ansiedad en la adolescencia válidos y fiables, que permitan caracterizar diferentes condiciones a lo largo del continuo de ansiedad. Para llevar a cabo este objetivo, se realizó un estudio meta-analítico de la literatura científica (Estudio 1) y tres estudios empíricos. El primer estudio empírico (Estudio 2 de la tesis) se centraba en el dominio subjetivo (mediante autoinformes), mientras que en los otros dos (Estudio 3 y 4 de la tesis) el principal foco de interés fue la búsqueda de biomarcadores fisiológicos cardiacos. Como objetivo secundario, se pretendía aportar una intervención preventiva basada en la evidencia para reducir la sintomatología ansiosa en la adolescencia y poner a prueba la eficacia de la

misma. Para este objetivo, se llevó a cabo el Estudio 5. Como principales resultados derivados de estos estudios, en el Estudio 1 se indicaron diferentes medidas no lineales provenientes de la TSD sensibles para la identificación de individuos con TA (entropía muestral o la dimensión fractal en los sistemas cerebral, cardiaco y hormonal). En relación al Estudio 2, se describió el curso natural de la sintomatología ansiosa desde la adolescencia temprana a la media, destacándose la influencia dinámica de varios factores de riesgo (género y factores temperamentales). En los Estudios 3 y 4, se observaron varios biomarcadores cardiacos para diferentes condiciones de ansiedad subclínica: el exponente alométrico *h* en contextos ecológicos (Estudio 3) y la entropía muestral cuando los adolescentes eran expuestos a un paradigma de laboratorio de inducción de estrés (Estudio 4). Por último, en el Estudio 5 se observó que el programa preventivo utilizado cortaba la trayectoria creciente de sintomatología ansiosa que se observó en algunos adolescentes. Para concluir, los estudios realizados dentro de esta tesis sirvieron para identificar diferentes marcadores (subjetivos y cardiacos) que caracterizan a individuos con diferentes condiciones desadaptativas en el continuo de la ansiedad. Estos marcadores fueron identificados desde una perspectiva flexible combinando aspectos madurativos y psicopatológicos. De dichos resultados, se desprenden algunas implicaciones prácticas para la evaluación y tratamiento clínicos a favor de instaurar protocolos más personalizados y promover políticas de salud preventivas para facilitar un desarrollo socioemocional más saludable en la adolescencia.

RESUM

L'ansietat constitueix un dels principals problemes de salut pública en les societats actuals donat el seu impacte econòmic i en la vida diària. Els actuals manuals diagnòstics tracten aquesta problemàtica mitjançant els anomenats trastorns d'ansietat (TA). Desafortunadament, moltes condicions ansioses amb important afectació del benestar personal no arriben al nivell de trastorn i, per tant, no es tenen en compte com a objectes de tractament. Per això, cal incorporar sistemes d'estudi més flexibles i no categorials. En aquest sentit, caldria destacar la iniciativa dels Criteris del Domini de Recerca (CDR) pel seu enfocament multidimensional (no estàtic ni categorial) en l'estudi de l'ansietat. Aquest enfocament considera condicions clíniques y subclíniques que causen malestar significatiu. La iniciativa CDR és especialment indicada per a l'estudi de l'ansietat en l'adolescència, principalment perquè els problemes d'ansietat en aquest període vital, fins i tot a nivells subclínics, són predictors significatius del desenvolupament de diferents problemes mentals en l'edat adulta (trastorns depressius, trastorn d'abús de substàncies, etc.). Per això, resulta crucial adoptar una perspectiva multidimensional en la cerca de marcadors d'ansietat en l'adolescència que dirigeixin la pràctica clínica. La Teoria dels sistemes dinàmics (TSD) pot contribuir significativament a aquest propòsit, en virtut de les evidències trobades des d'aquesta perspectiva per a la detecció de marcadors socioemocionals i fisiològics en individus amb diferents condicions psicopatològiques. En aquesta tesi doctoral es pretenia contribuir a la cerca de marcadors vàlids i fiables d'ansietat en l'adolescència, que permetin caracteritzar individus que experimentin diferents condicions al llarg del continu d'ansietat. Per tal d'assolir aquest objectiu, es va realitzar una meta-anàlisi de la literatura científica (Estudi 1) i tres estudis empírics. El primer estudi empíric (Estudi 2 a la tesi) es centrava en el domini subjectiu (mitjançant autoinformes), mentre que en els altres dos (Estudi 3 i 4 a la tesi) el principal focus d'interès era la cerca de biomarcadors fisiològics cardíacs. Com a objectiu secundari, es pretenia aportar una intervenció preventiva basada en l'evidència per reduir la simptomatologia ansiosa en l'adolescència i posar a prova l'eficàcia de la mateixa. Amb aquest propòsit es va dur a

terme l'Estudi 5. Com a principals resultats d'aquests estudis, a l'Estudi 1 es van trobar diferents mesures no lineals derivades de la TSD sensibles per a la identificació d'individus amb TA (l'entropia mostral o la dimensió fractal als sistemes cerebral, cardíac i hormonal). En relació a l'Estudi 2, es va descriure el curs natural de la simptomatologia ansiosa des de l'adolescència primerenca a la mitjana, destacant-se la influència dinàmica de varis factors de risc (gènere i factors temperamentals). Als Estudi 3 i 4, es va observar varis biomarcadors cardíacs per diferents condicions d'ansietat subclínica: l'exponent alomètric *h* a contextos ecològics (Estudi 3) i l'entropia mostral a contextos controlats d'inducció d'estrès (Estudi 4). Finalment, a l'Estudi 5 es va observar que el programa preventiu utilitzat va detenir la trajectòria natural creixent que van mostrar alguns adolescents en relació a la simptomatologia ansiosa. Per concloure, els estudis duts a terme dins d'aquesta tesi van servir per identificar diferents marcadors (subjectius i cardíacs) que caracteritzessin a individus amb diferents condicions al continu de l'ansietat. Aquests marcadors van ser identificats des d'una perspectiva flexible que combinava aspectes maduratius i psicopatològics. D'aquests resultats se'n deriven algunes implicacions pràctiques per a l'avaluació i tractament clínics a favor d'instaurar protocols més personalitzats i promoure polítiques de salut preventives per a facilitar un desenvolupament socioemocional més saludable en l'adolescència.

Background and General Framework 1

1.1. Anxious Emotion

Emotion is one of the most important biological components responsible for environmental adjustment in mammals. Due to its adaptive nature, emotion provides a wide repertoire of reaction and response patterns enabling species to maximize their chances of survival. Emotion is characterized by a strong gene signature which, just after birth, covers confrontation with a wide range of situations typically experienced throughout the lifespan of human beings. However, emotion-related patterns of behavior and feelings constantly interact with the environment and can be modified by learning and maturation (Barlow, 2002; Lang, Bradley, & Cuthbert, 1998).

Peter J. Lang and other theorists have differentiated emotions according to their motivational role in pursuing survival goals (see Konorski, 1967; Lang, 2010). Thus, they distinguish between appetitive emotions, which intend to satisfy the needs involved in species preservation (e.g., joy, sexual passion, etc.), and defensive emotions, those aimed at protecting individuals from noxious stimuli and activating defensive responses to ensure survival. Fear and related emotions, such as anxiety, are considered defensive as they trigger the initiation of chains of responses to avoid or escape threatening stimuli.

Traditionally, emotion has been studied through its three components (Barlow, 2002; Lang, 2010): the expressive-evaluative component, which covers all appraisals and cognitive perceptions (for human beings) that enable individuals to see the motivational properties of a stimulus (whether the stimulus may elicit either appetitive or defensive emotions); the physiological component, understood as the component triggering all the required bodily reactions to express a specific emotion (e.g., face blushing, the top-down release of corticotropines to trigger an escape response, etc.); and finally, the behavioral component, involved in the observable responses towards the stimulus and emotion-specific (e.g., approach, avoidance, freezing, etc.). The interaction

between all of these components is well known and shows a clear adaptive function to the extent that it can modulate the directional and strength aspects of emotion.

From an evolutionary standpoint, anxiety cannot be separated from fear. In fact, both emotions share some common features. Many scientists and theorists have discussed the overlap of both emotions as they both essentially function as an organism's alarm trigger involved in reactions to potentially survival-threatening stimuli. Barlow (2002) has made a major effort aimed at distinguishing between fear and anxiety. He considered fear as a basic, fundamental and discrete emotion, and anxiety as a more diffuse and imprecise one. Furthermore, fear would be more closely related to present or imminent danger and anxiety to preparing for upcoming potentially dangerous situations. Thus, stimuli that elicit fear-related reactions are often clearly identified (e.g., a spider, a major storm, darkness, etc.) and concrete behaviors often are exhibited in response to these stimuli (basically, fight, freeze or flight). Conversely, stimuli that elicit anxiety-related reactions are more diffuse (outdoor places, negative social scrutiny, etc.) and sometimes definite behaviors to face up to them are highly complex (e.g., the so-called active or passive chains of avoidant behavior). In turn, the physiological mechanisms involved in both emotions are relatively similar, although fear-related reactions require higher resources and sympathetic arousal to encourage responses to imminent danger (American Psychiatric Disorder [APA], 2013; Craske et al., 2009; McTeague & Lang, 2012; Thayer & Sternberg, 2006).

1.2. Maladaptive Anxiety as an Endemic Health Hazard in the 21st Century

In spite of the adaptive value of anxiety (and emotions in general), when it becomes either persistent or chronically activated in certain contexts, or is accompanied by very high physiological arousal, it becomes distressing and maladaptive, and tends to interfere with context adjustment (Nutt, Garcia-de Miguel, & Davis, 2008). Weems (2008) conceptualized maladaptive anxious emotion, defining it as a dysregulation in the normative anxiety response system in contexts and over time. Undoubtedly, some

deviation from normative patterns of expression is often adaptive and useful to enable individuals to develop better strategies of contextual adjustment. Nevertheless, when non-normative expressions of anxiety are linked with personal distress and functional interference on a daily basis, an anxiety disorder (AD) may arise.

ADs have been proven to be *high prevalence disorders* and their impact has been largely observed worldwide (Bandelow & Michaelis, 2015). For this reason, AD should be identified as an actual public health hazard. It is therefore crucial to have an accurate picture of how many people suffer from an AD in order to estimate the human economic burden, as well as the infrastructures needed to cope with the damaging effects of all the disorders. In this regard, it has made major strides to provide some sources of information, for instance by conducting prevalence or epidemiological studies that have been proven to be valuable for healthcare providers and decision-making boards alike.

As varying methods can be used in epidemiological studies, differences in prevalence and incidence rates of AD between studies have been observed. In this vein, it is common to find studies conducted with community samples and also in clinical settings. Furthermore, diagnostic criteria to establish clinical diagnoses have varied between studies: estimates of suffering an AD in the past year range from 8.4-25% (Bandelow & Michaelis, 2015; Copeland, Angold, Shanahan, & Costello, 2014; Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Conversely, lifetime prevalence rates point to 14-34% of the population suffering an AD at some point in their lifetime. Baxter, Scott, Vos and Whiteford (2013) performed an exhaustive systematic review and meta-analysis gathering data from epidemiological studies covering the five continents. They indicated that global prevalence rates of currently experiencing an AD (with the last three months at most) pointed to 7.3% of the population (ranging from 4.8-10.9%) and around 11.6% of the population in the last year (ranging from 7.6-17.7%). In Spain, a recent study found that the 12-month prevalence rate of suffering an AD during the period of the economic crisis (2010-2012) was around 9.7% (Navarro-Mateu et al., 2015).

Some sociodemographic factors may play a crucial role in how ADs are distributed among the population, as well as a world region (Bandelow & Michaelis, 2015; Baxter et al., 2013; Beesdo-Baum & Knappe, 2012; Kessler et al., 2012). In this vein, the bulk of studies point to higher rates in women in comparison to men (almost a third of women suffer from an AD in their lifetime).

In terms of resource use and economic burden, only a small proportion of people with AD receive mental health services. For instance, suffering an AD may represent almost 1% of emergency department visits, most being due to a panic attack (Dark, Flynn, Rust, Kinsell, & Harman, 2016; Essau & Gabbidon, 2013). Although direct costs derived from AD are close to €1000-1500 per person per year, they may reach €5000 in severe cases. The indirect costs from AD are even more significant, with high rates of school and work absenteeism, and lower productivity being observed (Bandelow & Michaelis, 2015; Konnopka, Leichsenring, Leibing, & Konig, 2009; Stuhldreher et al., 2014).

1.2.1. Traditional Categorical Conceptualization of Maladaptive Anxiety: Anxiety Disorders

Anxiety disorders have traditionally been studied as complex syndromes with varying components and signs of disease. In keeping with the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V; APA, 2013), an AD is characterized by a pattern of excessive fear and anxiety with subsequent behavioral disturbances. This pattern should be observed for at least six months as provoking a clear interference in the individual's areas of functioning as well as significant emotional distress. By and large, an anxiety-related or phobic stimulus, or a variation thereof, is the basis of any AD. Facing the associated phobic stimulus is therefore perceived to involve catastrophic consequences for the individual and as a result, the individual employs avoidant or fleeing behavior. Finally, reactions observed in the manifestation of an AD cannot be

linked to any other mental or organic disorder, or derived from taking psychoactive drugs.

Before the DSM-V was published a few years ago, the traditional classification of mental disorders covered eight main ADs (see the last edition of the DSM, the DSM-IV, revised text; APA, 2000). The so-called specific phobias are the simplest or most related to fearful feelings, where phobic stimuli are highly specific (for instance, fear of heights, fear of small insects, etc.). More complex phobic stimuli can be identified for social anxiety disorder (or social phobia), where a fear of a negative social appraisal or of being ridiculed within social settings leads to anxious symptomatology. Another social-related anxiety disorder, but highly linked with infancy, is selective mutism characterized by a failure to speak in social situations. Panic-related symptomatology may arise where the phobic stimulus derives from one's own bodily sensations, especially as a result of catastrophizing arousal-derived bodily reactions appraised as a sign of disease, a heart attack, etc. An agoraphobic set of symptoms may accompany panic symptomatology, where related unpleasant bodily reactions or catastrophic consequences from not being helped are associated with specific physical places (frequently, outdoors or public places). A special mention must be made of separation anxiety. This disorder is characterized by inappropriate fear and excessive anxiety linked to separation from major attachment figures. Until recently, this disorder had been labelled as a syndrome whose initial diagnosis came in infancy, childhood, or adolescence. However, the age-of-onset criterion for separation anxiety has been removed in the DSM-V, as it is now considered to be a condition that may span an entire lifetime (see Carmassi, Gesi, Massimetti, Shear, & Dell'Osso, 2015).

Generalized anxiety disorder (GAD) is even more diffusely conceptualized. Indeed, many authors and clinicians agree that this disorder is characterized by a fear of (almost) everything. Thus, a pattern of anxious apprehensiveness is described for a wide variety of life's areas (family, work, health, etc.) and, as a result, a heightened but moderate physiological arousal is displayed in GAD patients provoking acute physical tension and headaches, among other anxiety-related signs. Worrying is a typical

expression of maladaptive repetitive thinking and is the most common symptom in GAD (see Watkins, 2008).

The DSM-V also conceptualizes that an anxiety disorder may arise as a consequence of either an organic disease or a medical condition (e.g., pheochromocytoma), or as a consequence of substance abuse or medication (occasionally or repeatedly). Finally, the manual mentions other specific, sometimes culture-related, anxiety disorders (e.g., *khyal* or "wind attacks", and "attack of nerves") and a descriptor for unspecified AD (those characterized by anxiety symptomatology and causing significant functional impairment that do not fit with the aforementioned AD).

The transition of diagnostic entities from the DSM-IV, revised text (this manual has been followed throughout this dissertation as it was in use when the reference framework of the current doctoral dissertation was drawn up) to the DSM-V is worth noting. Obsessive-compulsive disorder (OCD) and posttraumatic stress disorder (PTSD) have always been considered part of the AD category. In this light, OCD comprises a set of symptoms characterized by recurrently experiencing repetitive thought manifestations, intrusive thoughts or obsessions (see Watkins, 2008); and/or the exhibition of behaviors to either neutralize the potential consequences of these thoughts or reduce levels of anxiety/physiological arousal. Some valuable recent research aimed at clarifying the neuroanatomical and genetic bases of OCD has led to consider this entity within the so-called obsessive continuum alongside other disorders (see, for instance, Hollander, Kim, Braun, Simeon, & Zohar, 2009). As a result of this, OCD has become part of a new, independent diagnostic category in DSM-V (the so-called Obsessive-compulsive and related disorders category). In turn, PTSD is a syndrome characterized by the reactions derived from either confronting or being exposed or a witness to a (very) stressful, even traumatic, event. Due to its similarities with other stress-related entities (Friedman, Resick, Bryant, & Brewin, 2011; Friedman, Resick, Bryant, et al., 2011), it has been incorporated into another independent DSM-V category (the so-called Trauma and stress-related disorders).

1.2.2. New Perspectives on the Study of Maladaptive Anxiety: The Research Domain of Criteria (RDoC) Initiative

The majority of studies examining how many people are suffering from an AD is focused on the so-called full-blown syndromes. This means a disorder should be fully manifested and the key diagnostic criteria considered as a source of distress in general terms. However, there are many highly distressing pathological conditions even where a mental disorder diagnosis cannot be considered at all. Moreover, and based on intervention studies, diagnostic categories are not as strongly correlated as expected with treatment selection and prognosis. For instance, two patients with a similar diagnosis of agoraphobia and panic disorder may respond differently to the same treatment (e.g., cognitive therapy) even when a very similar set of signs and pathological manifestations has been identified. Physiological and neural circuitry issues may be behind these divergent responses since the same sign may stem from varying mechanisms (e.g., an intrusive thought may be related to having experienced a traumatic event, for instance, or to be part of an obsessive-compulsive behavioral repertoire). Finally, recent findings support the necessity to consider an underlying general psychopathology substrate across mental disorders (see Caspi et al., 2014; Nivard et al., 2017; Van Os, 2013). This evidence may point to how tricky is to establish criteria-based, unitary diagnoses in mental health.

These circumstances have led many researchers to support a different method for describing and classifying the mental sources of distress. The current diagnostic systems (which the traditional manuals from the APA and the World Health Organization use as a basis) are strongly built around observable signs and symptoms. Therefore, their accuracy is poor since pathophysiological mechanisms and treatment responses are scarcely taken into consideration. In 2009, the National Institute of Mental Health launched the Research Domain Criteria (RDoC) Initiative (see Insel, 2014; Insel et al., 2010). The RDoC aims to provide precision for mental health sciences so as to construct a diagnostic system based on a deeper understanding of biological and psychosocial bases. This step-by-step initiative currently intends to be a framework for research

guidance towards the clarification of mental disorders, discarding the traditional conceptualization of categories or diagnostic entities, even though research may be based on current symptom-related syndromes as a starting point.

Thus, the RDoC aims at obtaining biomarkers relevant to guide modern mental health diagnosis and treatment selection. Five key domains of analysis have been established from the RDoC in the search for these biomarkers, so as to make an integrative picture on how an entity of distress may be represented (see https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml):

Negative valence systems

Positive valence systems

Cognitive systems

Social processes

Arousal and regulatory systems

Every domain covers some units of analysis: genes, molecules, cells, circuits, physiology (well-established indices of certain constructs; for instance, heart rate variability or cortisol release in anxiety), behaviors (observed patterns of responses exhibited in ecological contexts or when confronting a behavioral task), self-reports and paradigms (well-established protocols to study a process or entity).

The RDoC initiative is currently guiding research in the field of anxiety and, accordingly, relevant findings have been obtained. Simpson (2012) has mentioned, for instance, that patients with GAD and other AD (e.g., social phobia) show some evidence of stimulus overgeneralization when fear theories are used as a reference framework (in a negative valence systems domain). She has also cited that all patients with AD exhibit defensive hyperarousal during affective challenge and attentional

biases (some evidence from cognitive and regulatory systems). Finally, she has highlighted the neural mechanisms of cognitive control deficits which were linked with dopamine receptor D2 dysfunction in AD patients (cognitive systems domain). Nusslock, Walden, and Harmon-Jones (2015) have proposed asymmetrical frontal cortical activity as a biomarker for the anxious apprehension characteristic of AD and also of mood disorders.

In terms of developmental psychopathology, Casey, Oliveri and Insel (2014) have postulated how important it is to integrate crucial concepts of development into the research of RDoC key domains. First, these authors have highlighted the importance of taking into account the developmental trajectory of some signs or manifestations of a disorder over a lifespan. This trajectory may depict a linear or nonlinear course with different peaks across life periods. These peaks may represent sensitive periods or restricted windows of development, characterized by a greater susceptibility to influence from the effects of external and internal risk factors. For instance, in terms of specific ADs, a developmental course of social anxiety symptomatology is shown that may emerge in childhood, reach a peak of highest manifestation throughout adolescence and steadily decrease throughout adulthood and among the elderly. Other ADs, such as generalized anxiety symptomatology may reach its highest levels in youth and adulthood (Bandelow & Michaelis, 2015; Kessler et al., 2012; McDowell et al., 2014). Finally, Casey et al. have mentioned the dynamic interaction of systems throughout development. Brain circuits and physiological systems interact dynamically throughout a lifespan leading to constant changes in context adjustment. Thus, a deficit occurring early in development may lead to a cascade of more complex deficits as brain and physiological systems mature and keep interacting over time. Accordingly, a risk factor may present multifinality (a single risk factor may constitute a risk of multiple disorders) and its interaction with internal and external conditions may lead to the development of varying sources of suffering (Garvey, Avenevoli, & Anderson, 2016; van Os, 2013).

1.2.3. Maladaptive Anxiety as a Subclinical Syndrome

1.2.3.1. Subclinical Anxiety and Human Suffering

Along with perspectives calling for a reconsideration of the taxonomic and rigid classifications of mental disorders, some evidence has highlighted the relevance of subthreshold syndromes. A subthreshold or subclinical disorder can be defined as a distressing entity covering a bulk of symptoms that characterize a full-blown disorder. However, and according to traditional diagnostic manuals, the absence of one or several symptoms means full diagnostic criteria are not met, or the criterion for duration is not fulfilled (Okasha, 2009). Distress due to a subclinical disorder, as well as the prevalence rates and its functional interference, are often equivalent to those of full-blown disorders (Balasz et al., 2013).

Many examples can be provided in this regard. Rucci et al. (2003) found that individuals who attended primary care services suffering from subclinical mental disorders showed increased psychological distress, daily disability and poorer health perception in comparison to health controls. In terms of PTSD, a similar level of distress and impairment (based on the lack of between-group differences) was described when comparing full-blown and subclinical PTSD patients (Mota et al., 2016; Zlotnik, Franklin, & Zimmerman, 2002). Prevalence rates of subclinical disorders are worth noting. Thus, when comparing patients with a subclinical or a full-blown disorder, all with significant impairment, the prevalence rates of bipolar-related disorders may raise at least fivefold when comparing to the traditionally defined diagnostic criteria (see Berk et al., 2008). In turn, approximately a half of adolescents may meet the criteria for full and/or subclinical depression and/or anxiety (Balasz et al., 2013). More specifically, and considering mood symptomatology, Merikangas et al. (2007) found that only about 15% of the population reported no such symptoms (subclinical or full-blown depression or hypomania) over their lifetime, thus exhibiting these symptoms could be termed “normal”.

Taking this and other evidence together, subclinical and full syndromic disorders may be viewed as falling along a continuum, with subclinical disorders being considered as quantitatively lower than full-blown disorders, but qualitatively distinctive (Okasha, 2009; Shankman, Klein, Lewinsohn, Seeley, & Small, 2008; Zammit et al., 2013). Nonetheless, traditional systems of mental disorder classification have no place for subclinical disorders except in atypical, not otherwise specified entities (Okasha, 2009).

Subclinical anxiety disorders do not fully meet diagnostic criteria but show high levels of disability and are related to clinical symptomatology and impairment (Fehm, Beesdo-Baum, Jacobi, & Fiedler, 2008; Karsten et al., 2011; Roberts, Fisher, Turner, & Tang, 2015). Moreover, they are very common among the general population and continue to increase (Burstein, Beesdo-Baum, He, & Merikangas, 2014; Rucci et al., 2003). Subclinical GAD is highly persistent and twice as commonly suffered as full-blown GAD (Haller, Cramer, Lauche, Gass, & Dobos, 2014). Moreover, the presence of subclinical GAD is highly related to psychosocial and work interference, alongside high primary healthcare use.

Experiencing a subclinical anxiety disorder usually leads to the development of the full syndrome (Balasz et al., 2013; Shankman et al., 2009). Karsten et al. (2011) have found that subthreshold anxiety or depression, as well as prior full-blown episodes of these entities, were predictors of a new episode of the related emotional disorder two years later. Furthermore, the combination of subthreshold levels of anxiety and depression carried the highest risk for emotional disorders. Finally, a history of depressive disorder and subthreshold depression signaled the occurrence of either depressive or anxiety disorder; whereas a history of anxiety disorder or subthreshold anxiety signaled the occurrence of anxiety alone.

All the evidence set out in this chapter highlights that subclinical entities should be considered as different and distressing points along the continuum of anxiety, not only because they exhibit the same symptomatology as full-blown disorders at a lower level but also because they show distinctive features which make them characteristic and far beyond the categorical labels used in traditional diagnostic manuals.

1.2.3.2. Risk Factors for Maladaptive Anxiety

Many researchers have been interested as to why a mental disorder (perhaps an anxiety disorder) develops in some individuals and under certain conditions, but not in others. For instance, different circumstances may be the trigger for developing PTSD (e.g., a situation of social exclusion). However, not all individuals develop this syndrome. In this regard, the influence of some factors, so-called risk factors, may play a crucial role on the manifestation of a specific disorder. According to the World Health Organization (WHO), a risk factor is "any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury" (WHO, 2017). In the context of ADs, a risk factor would be an intrinsic or extrinsic condition that makes the appearance of an anxiety disorder more likely.

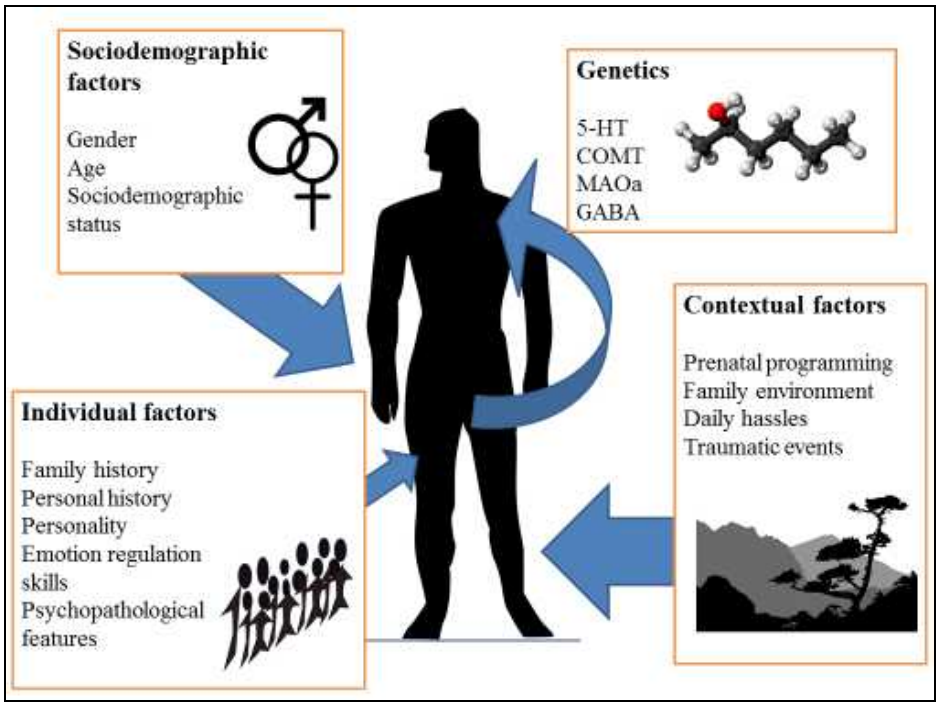
It is very difficult to identify how many risk factors may influence individual behavior, cognition and emotion at the same time, and how strong each influence is. Furthermore, a complex interaction between risk factors over time is put forward as being involved in the development of anxiety disorders and subclinical syndromes (Brook & Schmidt, 2008; Domschke & Reif, 2012). In this regard, theorists and researchers have to look at such problems in order to disentangle individual sources of risk to promote preventive and intervention programs. Therefore, some domains have been proposed so as to lead efforts for risk factor identification (see Figure 1).

Genetics

A significant heritability of anxiety disorders has been observed across studies. Thus, some evidence points to around 30-50% of the risk of developing an anxiety disorder is genetically predisposed (Clement, Calatayud, & Belzung, 2002; Drake &

Ginsburg, 2012; Gatt, Burton, Williams, & Schofield, 2015). By disorder, a heritability rate of about 32% has been estimated for GAD, being higher for social phobia (around 50%) and specific phobias (e.g., blood-injection phobia with a heritability rate of 59%). The cases of panic disorder and agoraphobia deserve special mention (see Domschke & Reif, 2012). In this regard, a heritability rate of 62% has been estimated for agoraphobia. In turn, there is a three to fivefold increased prevalence for being diagnosed with a panic disorder among individuals whose first-degree relatives also suffered from a panic disorder.

Figure 1. Main risk factor domains influencing anxiety.



Despite the high heritability rates found, only a relatively small number of genes may underlie the genetic risk for anxiety disorders. Moreover, it seems that the bulk of these genes are involved in the development of other mental disorders such as mood disorders or schizophrenia, as well as other pathological entities or risk factors (mental distress, neuroticism, harm avoidance, etc.). Thus, a strong link between anxiety and

some single nucleotide polymorphisms of several genes involved in the serotonin (5-HT) system has been found (Clement et al., 2002; Domschke & Reif, 2012; Savage, Sawyers, Roberson-Nay, & Hettema, 2017; Zavos, Eley, & Gregory, 2013), especially in the regulatory region of the serotonin transporter and 5-HT receptors (HTR1A, HTR2A, 5-HTTLPR, 5-HT1A, 5-HT1B, 5-HT2A). Furthermore, genes involved in the catecholamine systems (adrenaline, dopamine, etc.) also show some polymorphisms associated with maladaptive anxious emotion and the development of anxiety disorders: the catechol-O-methyltransferase (COMT) gene and monoamine oxidase A (MAOa), which play a role in catecholamine metabolism, are worth highlighting (Clement et al., 2002). Thus, for instance, a polymorphism of the Val allele of COMT (Val158met polymorphism) has been identified in individuals with panic disorder; whereas the Met allele seems to be related to OCD repertoires (see Gatt et al., 2015). Additionally, a polymorphism of the dopamine transporter (SLC6A3) gene seems to be involved in social phobia and GAD, while the DRD2 variants of the dopamine receptor R2 have been associated with the pathogenesis of PTSD (see Domschke & Reif, 2012). Other studies have shown the influence of receptor D3 and D4 genes.

Further genes have been linked with anxiety disorders and anxiety severity. The transmembrane protein 132D gene seems to be a candidate for anxiety phenotypes due to the strong relationships found with the severity of anxiety, anxiety trait and panic disorder (Erhardt et al., 2011; Gatt et al., 2015). Some genes of GABAergic receptors and transporters have been linked with anxiety disorders (Pham et al., 2009; Thoeringer et al., 2009). Genes related to the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (e.g., CRF1 and CRF2 receptors) and inflammatory processes (e.g., C-reactive protein gene) are involved in susceptibility to ADs and to becoming more anxious (Clement et al., 2002; Luciano et al., 2010).

Some authors highlight the composite influence of showing some genes (the so-called polygenic risk) as an additional marker of being in a higher risk for AD (Savage et al., 2017; Santoro et al., 2016). Moreover, polygenic risk studies point to the genetic overlap as a reflex of the influence of the general psychopathology factor on definite disorders (see Caspi et al., 2014). Accordingly, some studies examining polygenic risk

of suffering some concrete disorders (e.g., psychotic disorders or mood disorders) have revealed a strong influence of that on the development of an AD (Nivard et al., 2017; So & Sham, 2017).

Sociodemographic Factors

There are three sociodemographic factors strongly linked with the development of anxiety disorders: gender, age and sociodemographic status. Other studies have made an important effort to elucidate the influence of other sociodemographic factors (e.g., educational level, race, etc.,) but their results remain highly inconsistent (Brook & Schmidt, 2008; Copeland et al., 2014; Grant et al., 2009; Moreno-Peral et al., 2014).

Gender, especially for females, has been widely linked with anxiety disorders and anxiety severity. Indeed, a 2:1 or even 3:1 female-to-male ratio has been observed regarding every AD (Beesdo-Baum & Knappe, 2012; Grant et al., 2009). Age also constitutes a key factor in the development of anxiety disorders to the extent that certain life periods are deemed critical (sensitive) to the expression of specific types of symptomatology (Casey et al., 2014; Copeland et al., 2014; Kessler et al., 2012; Weems, 2008). Thus, higher prevalence rates of GAD may be found in youth and early adulthood, with a greater manifestation of the fear of darkness in childhood. Furthermore, the highest levels of social anxiety symptomatology are reported during early and mid-adolescence. Finally, low socioeconomic status has often associated with the development of anxiety disorders, especially in the extreme extent of poverty (Brook & Schmidt, 2008; Hirshfeld-Becker, Micco, Simoes, & Henin, 2008; Moreno-Peral et al., 2014).

Individual Factors

This area embraces various key aspects of physical and mental health, as well as other individual-specific cognitive or behavioral factors. Strong support has been obtained from studies in terms of linkage with anxiety severity or the development of anxiety disorders.

Initially, it is important to consider the influence of a family's history of mental problems. Moreno-Peral et al. (2014) conducted a systematic review of cohort studies analyzing the role of various factors on anxiety disorders. They pointed out that patients with panic and GAD had more frequently witnessed parental depression, anxiety disorders or sleep problems, than healthy control individuals. Moreover, socially anxious children are more likely to have parents with social phobia at clinical or subclinical levels (Brook & Schmidt, 2008; Ferro & Boyle, 2015; Knappe, Beesdo-Baum, & Wittchen, 2010).

Likewise, the influence of an individual's prior history of mental disorders may also determine their presenting with anxiety disorders over their lifetime. Thus, anxiety and depressive symptoms may lead to the development of subsequent anxiety disorders (Balasz et al., 2013; Karsten et al., 2011; Shankman et al., 2009). Additionally, other disorders, such as sleep or bipolar disorders, may be associated with the development of anxiety disorders over time (Brook & Schmidt, 2008; Moreno-Peral et al., 2014). In turn, some physical conditions have also been linked to anxiety severity and the development of ADs: pheochromocytoma, joint hypermobility syndrome and carbon dioxide (CO_2) hypersensitivity have been directly linked with panic disorder (APA, 2013; Leibold et al., 2016; Moreno-Peral et al., 2014).

Alongside how a similar prior disorder or others may gradually lead to the development of an anxiety disorder, one distinction should be taken into consideration: homotypic and heterotypic continuity (Hirshfeld-Becker et al., 2013; Ferdinand, Dieleman, Ormel, & Verhulst, 2007). When an anxiety disorder shows homotypic continuity over time, this disorder would be expressed at a particular moment in life

(e.g., childhood), then remit and arise over time. Some stability or chronicity is assumed to be behind the homotypic continuity principle, given the symptomatic expression fluctuating throughout life (Seligman & Gahr, 2013). Conversely, heterotypic continuity would be seen when a specific disorder emerges as a result of the prior influence of others. In other words, a secondary (anxiety) disorder may be developed as a result of a primary one over time. Mixed evidence supports how both mechanisms lead to the development and course of anxiety disorders. There is some common overlap between anxiety disorders, affective disorders and others (e.g., sleep problems and eating disorders) from a genetic and phenomenological standpoint (Gatt et al., 2015; Seligman & Gahr, 2013; Trzaskowski, Zavos, Haworth, Plomin, & Eley, 2011; Waszczuk, Zavos, Gregory, & Eley, 2016). As Van Os states:

"The earliest expressions of psychopathology are a nonspecific, mixed bag of affective dysregulation, aberrant salience, motivational alterations, anxiety states, and other early symptoms that dynamically affect each other, forming a causal network." (van Os, 2013, p. 696).

Van Os suggested the presence of a general psychopathology factor (in line with Caspi et al., 2014) and the necessity to consider the potential genetic and symptomatic overlap among mental health entities. Nevertheless, it is highly likely that the presence of a prior specific disorder may lead to the manifestation of the same one over time (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Ferdinand et al., 2007; Newman, Shin, & Zuellig, 2016). For instance, in a study conducted with adolescents (de la Torre-Luque, Balle, Fiol-Veny, Llabres, & Bornas, under review), more than 70% of participants were found to show the same levels of anxiety after completing two assessments with an interval of six months between assessment points. This points to some stability of symptomatology over this period.

When it comes to other key aspects in the development of anxiety disorders, it is worth noting the influence of personality (and its developmental precursor,

temperament) and the strategies of emotion regulation as risk factors. These factors have not always been deemed as noxious or pathological as the aforementioned factors. Indeed, they may sometimes be adaptive, although when interacting with genes and the environment they may lead to the development of ADs.

In terms of personality, some factors have been strongly linked to anxiety, such as high behavioral inhibition, harm avoidance, neuroticism, shyness and negative affectivity; as well as low conscientiousness, effortful control (EC) and extraversion (de Pauw & Mervielde, 2010; Hirshfeld-Becker et al., 2008; Kotov, Gamez, Schmidt, & Watson, 2010; Rothbart, 2007). Many of these constructs may explain relatively similar traits albeit from different frameworks (see Nigg, 2006): for instance, neuroticism from the Big Five framework would be analogous to negative affectivity from Rothbart's framework. Also, it is important to mention the role of anxiety sensitivity (AS) as a trait-like cognitive risk factor. AS refers to an apprehension to anxiety-related bodily sensations due to the potential harmful consequences that they might have (Reiss & McNally, 1985). Strong evidence has been obtained in terms of the relationship between higher anxiety severity and AS (see Olatunji & Wolitsky-Taylor, 2009). Self-esteem also is associated in a negative way with anxiety symptomatology and the development of an AD (Ferro & Boyle, 2015; Van Tuijl, de Jong, Sportel, de Hullu, & Nauta, 2014).

Finally, it is very important to highlight how the strategies individuals use to deal with difficult situations impact the development of anxiety disorders. Emotion regulation comprises a set of heterogeneous actions that intends to influence "which emotions we have, when we have them, and how we experience and express them" (Cisler, Olatunji, Feldner, & Forsyth, 2010). Emotion regulation may boost the effect of emotional reactivity on anxiety disorders or be involved in a cumulative effect of some risk factors on their development. In this regard, the use of daily repetitive thinking, particularly rumination, strategies (see Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008; Watkins, 2008), seems to heighten the severity of anxiety symptomatology and influences the coping with stressful or traumatic events (Brosschot, 2010; Michl, McLaughlin, Sheperd, and Nolen-Hoeksema, 2013; Tortella-Feliu et al., 2014). In fact, worry as a strategy of emotion regulation turns into the key feature of GAD. Other

strategies such as avoidant-like strategies (e.g., suppression) and negative re-appraisal have also been linked with higher anxiety severity (Aldao, Nolen-Hoeksema, & Schweizer, 2010; Berking & Whitley, 2014; Cisler & Olatunji, 2012).

Contextual Factors

Current perspectives examining the development of psychopathology postulate that contextual factors may play a significant role in conjunction with genes and individual-specific aspects (see Barlow, 2002). Diverse events or contextual circumstances may determine how and when an anxiety disorder is expressed.

Starting early in development, it is important to highlight the influence of prenatal events on the subsequent development of ADs. Thus, high levels of psychosocial stress during pregnancy may often bring about some health outcomes, such as lower birthweight or prematurity, as well as psychiatric symptomatology, such as internalizing disorders (Glover, 2011; Huizink, 2012). Some theories have identified the influence of heightened prenatal levels of cortisol on the development of these health outcomes (Zijlmans, Riksen-Walraven, & de Weerth, 2015). Traumatic events experienced by the pregnant mother may also amplify the expression of anxiety-related symptoms across the lifespan (King, Dancause, Turcotte-Tremblay, Veru, & Laplante, 2012; Self-Brown, Lai, Harbin, & Kelley, 2014).

Over the course of life, some substantial evidence supports family-related styles of attachment and rearing having a decisive impact on the development of anxiety symptomatology in childhood and adolescence. In fact, family environment might be even more predictive for a child's mental health than any parental history of mental disorders. Thus, a strong association between insecure attachment and anxiety disorders or higher anxiety severity has been described in several studies (Brook & Schmidt, 2008; van Oort, Greaves-Lord, Ormel, Verhulst, & Huizink, 2011). Moreover, lower

levels of parental emotional warmth, higher levels of parental overprotection and rejection have been associated with child social phobia (Knappe et al., 2010).

Other events with higher emotional impact (e.g., experiencing a traffic accident, witnessing domestic violence, etc.) dramatically heighten anxiety symptomatology and the onset of anxiety disorders, with PTSD being more closely linked to having experienced these events (Brook & Schmidt, 2008; Grenier et al., 2015; Hashoul-Andary et al., 2016; Moreno-Peral et al., 2014). In this regard, it is important to highlight the role of traumatic events within the family setting. A parental divorce or the loss of a parental figure, or both, leads unavoidably to the development of some acute stress disorder, characterized by depressive and anxiety symptomatology, among others (Mueller, Baudoncq, & de Schryver, 2015; Muris & Ollendick, 2015; Otowa, York, Gardner, Kendler, & Hettema, 2014). Instances of physical and sexual abuse lead to even worse consequences with some authors stating that they are devastating and profoundly distressing in terms of psychological and physiological impairment (Banihashemi, Sheu, Midei, & Gianaros, 2015; Ip et al., 2016; Lindert et al., 2014). Additionally, some peer-related events are closely linked to the development of anxiety disorders: the cumulative effects of unsuccessful peer relationships (e.g., recurrent attempts to make friends or date someone), teasing and bullying. Severe and traumatic bullying seems to clearly lead to anxiety symptomatology, especially social anxiety or PTSD, given the negative social interaction between the bully and victim (Brook & Schmidt, 2008; Nielsen, Tangen, Idsoe, Matthiesen, & Mageroy, 2015).

Finally, the influence of culture on the manifestation of anxiety disorders should be noted and some forms of anxiety symptomatology have been described (Brook & Schmidt, 2008). For instance, a syndrome known as “attack of nerves” has been described among Latino descents, characterized by severe symptoms of emotional distress, such as acute anxiety and panic, anger, and shouting uncontrollably (APA, 2013); and the *taijin kyofusho* syndrome, observed among Japanese people, is conceptualized as a subtype of social phobia and characterized by an intense fear of offending others.

1.3. Markers of Maladaptive Anxiety: State of the Art

Researchers and clinicians have attempted to provide different tools that enable the complex phenomenon of anxiety to be disentangled, as well as signs of impairment in the emotional and physiological systems to be identified. Alongside a classical tradition in health sciences, and covering two of the RDoC units of analyses, two main areas can be considered in the search for markers of maladaptive anxiety in humans: one (the subjective domain) based on reports from individuals and one (the objective domain) based on the physiological correlates of human processes. Evidence taken from the assessment in both domains turns out to be highly complementary and enables a more accurate picture of anxiety phenomenon to be made. Marker refers to subjective or objective (biomarkers) signs of a specific condition being present (in this instance, subclinical anxiety or AD). The concept would be relatively equivalent to phenotype or endophenotype but also include psychological or subjective elements that point to the presence of meaningful anxiety.

A collection of evidence derived from research in subjective and objective domains is set out in the following chapters.

1.3.1. Subjective Markers

Distress symptomatology is often reported by individuals suffering from severe anxiety at a subthreshold level or from an anxiety disorder. In fact, complaints about how hard or interfering it is to live with anxiety may constitute the first evidence for suspecting the existence of an anxiety disorder (Rose & Devine, 2014). For this reason, exploring the subjective domain becomes paramount for a reliable and accurate assessment of anxiety in community and clinical contexts.

Two types of instruments can be used to evaluate anxiety in the subjective domain, depending on the respondent. Thus, so-called hetero-reports constitute instruments to explore the individual's subjective domain by means of an interviewer or implementer (usually, a clinician or a researcher) asking the individual under assessment key questions. As a result, an impression on a specific syndrome or phenomenon behind their behavior or symptomatology can be made. The most usual hetero-applied reports are interviews and questionnaires. In turn, self-reports are another source for collecting data in the subjective domain and are filled out by the individuals under assessment themselves.

Both instruments have advantages and disadvantages, and their feasibility in terms of administration mainly depends on the context conditions of delivery and the population under analysis. For instance, when studying children, it is common to assess anxiety by combining data collected from self-reports and hetero-reports (e.g., analyzing parents' and teachers' points of view) to get an integrative and thorough picture on anxiety symptomatology, as well as to avoid individuals' misunderstandings. Self-reports are widely used to assess anxiety thanks to their easy administration and capability of safeguarding individual's intimacy while being assessed. However, self-report conclusions are influenced by some important threats, such as social desirability response bias, acquiescence bias, etc. (see Paulhus & Vazire, 2007; van de Mortel, 2008).

In this regard, some organizations have established quality standards in order to ensure valuable and reliable conclusions can be extracted from self-reports (see for instance, the Australian Psychological Society guidelines for the use of psychological tests, Australian Psychological Society, 1997; or the standards from the British Psychological Society, 2017). The International Test Commission (ITC) has proposed some guidelines for test use (ITC, 2001) and with regard to the psychometric properties that a test should have, has stated:

"Tests should be supported by evidence of reliability and validity for their intended purpose. Evidence should be provided to support the inferences that may be drawn from the scores on the test. This evidence should be accessible to the test user and available for independent scrutiny and evaluation." (ITC, 2001, p. 106).

Therefore, in order to select self-reports for anxiety assessment, it is highly recommended that some evidence of validity (i.e., some evidence pointing to a theoretical structure being preserved in self-reports) and high accuracy (i.e., appropriate levels of reliability) are ensured (see; Ellis, 2013; Rose & Devine, 2014). Moreover, self-report instruments should show adequate psychometrical propierties considering the way of administration (e.g., on the Internet, on a paper-and-pencil format) and within the study sample (see Elosua, 2003; Parshall, Spray, Kalohn, & Davey, 2002).

As stated above, a wide variety of valid and accurate self-reports to measure anxiety symptomatology and related constructs can be used. The simplest self-report is the visual analog scale, comprising a single item in order to measure the degree of anxiety severity (Hornblow & Kidson, 1976). Nonetheless, the majority of researchers and clinicians prefer using self-reports which capture varying features of the complex phenomenon of anxiety. The visual analog scales can be useful when assessment time is limited or when screening (see Davey, Barratt, Butow, & Deeks, 2007).

Higher scores (or scores pointing to higher levels of self-reported anxiety symptomatology or severity) have been seen in people with subclinical or clinical anxiety when administering self-reports (Chorpita, Moffitt, & Gray, 2005; Masia-Warner et al., 2003; Michopoulos et al., 2008). Moreover, anxiety self-reports are often sensitive to treatment outcomes and allow for symptom monitoring over time (Anderson et al., 2013; Bornas, Balle, de la Torre-Luque, Fiol-Veny, & Llabres, 2015; Bluett, Homan, Morrison, Levin, & Twohig, 2014; Nelemans et al., 2014; Oerlemans et al., 2014). These instruments are classified according to different key aspects. Three of these aspects are crucial in order to obtain valuable conclusions from the assessment. The first aspect is related to the population under assessment: instruments adapted to a

child, adolescent or adult population should be selected depending on the population of the study. Secondly, self-reports can be selected in order to explore global anxiety or any subtype of anxiety symptomatology. Finally, self-reports can be selected if a clinical or community sample is studied.

Likewise, other related constructs (e.g., anxiety trait, anxiety state, anxiety sensitivity, etc.) can also be measured by self-reports. Some evidence also supports that individuals suffering from subclinical or clinical anxiety syndromes may score higher in these instruments (Bentley et al., 2013; Tendais, Costa, Conde, & Figueiredo, 2014; Woud, Becker, Rinck, Harmer, & Reinecke, 2016).

1.3.2. Central Physiological Biomarkers

Along with the tradition of searching for objective markers of anxiety disorders, a distinction between central anxiety-related correlates (from cerebral structures and processes) and peripheral ones needs to be made. A major effort is made to gain knowledge about how brain structures may work and interact with one another to contribute to an integrated physiological process. Central physiological biomarkers can therefore be obtained by means of analyzing biochemical substances in the central nervous system, neuronal glucose uptake as a sign of a physiological system function (through functional neuroimaging studies) and brain electrophysiology.

Functional Neuroimaging Studies

More integrated knowledge has been gained with regard to the neuronal circuits and systems involved in the physiological processes of anxiety through functional neuroimaging techniques. Experimental protocols and tasks have been designed to elicit neuronal responses linked to the physiological process under analysis (e.g., emotional

information processing); examples of these tasks include the presentation of emotion-related images (for instance, human faces showing emotional expressions, phobic stimuli, etc.), symptom provocation paradigms, stress induction protocols, etc. All of these tasks are deemed to elicit an organism's phasic responses as the organism (brain, neuronal groups) responds under the influence of the task requirements.

Results from functional neuroimaging techniques have shown the alterations in brain systems involved in information processing and reward-directed behavior (Bandelow et al., 2016; Bas-Hoogendam et al., 2016; Damsa et al., 2009; Duval et al., 2015; Francati et al., 2007; Mochcovitch, Freire, Garcia, & Nardi, 2014; Nusslock et al., 2015). The main findings in this regard point to increased activations in the left posterior cingulum, prefrontal cortex and amygdala, and the right hippocampus when exposed to potentially threatening stimuli. The orbitofrontal cortex, and the anterior and posterior cingulate cortices also show increased activity after being presented with anxiety-related stimuli. When presenting faces with emotional expressions the results are slightly more complex: some low activation of the anterior cingulate cortex and amygdala has been observed with anxious faces, although some hyperactivation of cingulate cortex neurons (with no differentiated activation in the amygdala of anxious participants compared to healthy controls) has been seen with exposure to happy faces. For panic participants, greater metabolic activity has been observed in the insular cortices, left inferior frontal gyrus, dorsomedial prefrontal cortex, the left hippocampal formation and left caudatum when responses to panic or neutral pictures were compared.

With regard to GAD participants, increased metabolic activity has been found in the lateral and medial prefrontal cortices and anterior cingulate cortex after being presented with emotional faces, alongside a higher activation of the amygdala when exposed to neutral faces (Blair et al. 2008; Holzel et al., 2013; Palm, Elliott, McKie, Deakin, & Anderson, 2011). In terms of social phobia participants, Bas-Hoogendam et al. (2016) have stated four main alterations: a) a pattern of heightened reactivity to novel or salient stimuli in the amygdala, as well as changes in its connectivity network; b) a pattern of hyperactivation of the fear circuit (amygdala, insula, anterior cingulate

and prefrontal cortex); c) changes in whole-brain functional connectivity under resting conditions, as well as under phasic conditions, and d) hyperreactivity of the medial prefrontal cortex, which is highly connected to the fear of being negatively evaluated by others. Finally, findings in OCD individuals point to abnormalities in orbitofrontal-striatal neural circuitry, considered to be a mediator of the disorder, associated cognitive impairment and its clinical manifestations, as well as an increased activation in frontal and parietal areas (see Bandelow et al., 2016; Menzies et al., 2008; Milad & Rauch, 2012).

Neurochemical Dynamics and Anxiety

Neurochemical dynamics reflect how neurons communicate with one another when a definite physiological process is ongoing. Hence, the study of neurotransmission systems becomes crucial to delimit how altered physiological processes are in anxious participants. Studies on neurochemical dynamics comprise pharmacological trials consisting of analyzing how an organism responds to infusions of a neurotransmitter-related agonist or antagonist, clinical trials with pharmacological therapy, and laboratory assays examining the availability of neurotransmitter molecules (e.g., metabolites) or the density of neurotransmitter receptors within the extracellular space.

The neurotransmitter most closely linked to mood and anxiety disorders is serotonin (5-HT), which mediates the primary fight-or-flight response trigger when exposed to threatening stimuli (basically, stimuli that elicit fear). Changes in the concentrations of 5-HT, its metabolites in cerebrospinal fluid and the density of receptors have been reported in individuals with anxiety (see Bandelow et al., 2017; Damsa et al., 2009). Moreover, administration of serotonergic agonists (e.g., serotonin selective reuptake inhibitors) leads to ameliorations in panic and social phobia symptomatology to the extent that these drugs are considered first line treatments for these disorders (Bouwer & Stein, 1998; Furmark, 2009; Kimmel, Roy-Byrne, & Cowley, 2015).

The dopamine (DA) neurotransmission system has also been studied in the context of anxiety disorders. DA as a main common catecholamine within the brain plays a significant role in multiple physiological and cognitive functions, such as the reward motivational system, goal-oriented decision making and motor control. Studies analyzing the DA system have failed to identify differential patterns of neurotransmission regulation among phobic or panic individuals in comparison to healthy controls (see Bandelow et al., 2017; Damsa et al., 2009). Nevertheless, other studies support that dopamine alterations seem to play a significant role in social phobia, GAD and PTSD (Bandelow et al., 2017; Furmark, 2009; Tiihinen et al., 1997).

The neurotransmitter norepinephrine (NE) has been extensively linked to anxiety disorders, probably due to its connection to the HPA axis, also called the stress axis. There is a vast body of evidence suggesting that enhanced noradrenergic neurotransmission (especially in the hypothalamus, amygdala and locus coeruleus) might be associated with anxiety and agitation (Montoya, Bruins, Katzman, & Blier, 2016).

The role of the γ-aminobutyric acid (GABA) neurotransmission system deserves special attention. Some theories have proposed the emergence of anxiety and mood disorders as a consequence of dysfunctions in central GABA-mediated inhibitory mechanisms (Croarkin, Levinson, & Daskalakis, 2011; Goddard, 2016; Nuss, 2015). Some evidence in this regard is in line with the anxiogenic effects of benzodiazepines and the alterations observed in $GABA_A$ receptor sensitivity as mediators in the pathogenesis of panic attacks.

Other neurotransmission systems involved in the pathogenesis and maintenance of anxiety disorders (Bandelow et al., 2017) are: cholecystokinin, an excitatory messenger highly involved in appetite control and body weight that seems to play a significant modulating role on the neuronal networks of anxiety, especially in panic; central neurokinins, showing decreased NK_1 receptor binding in panic individuals or when exposed to a phobic stimulus; and, due to its link to social attachment, oxytocin has been observed as being closely involved in anxiolytic amygdala-mediated responses in social phobics or individuals with separation anxiety.

Electrophysiological Biomarkers of Anxiety

Various methods have been developed to measure the electrical potential of neurons based on non-invasive procedures. Electrical potentials in neurons are commonly taken by means of a set of electrodes attached to the scalp and distributed depending on the brain areas of interest. In this vein, two sets of techniques can be used to examine electrical signals from the scalp: electroencephalography (EEG) or magnetoencephalography (MEG) techniques; or evoked potentials (EP) or event-related potential (ERP) techniques. EEG and MEG recordings allow for a wider picture of how the brain works under resting or phasic conditions to be taken. When studying sleep, an EEG may be part of an integrated procedure, the so-called polysomnography (PSG) recording. In contrast, EP and ERP recordings are aimed at studying electrical activity in a reduced group of neurons to provide a detailed picture of their involvement in concrete physiological processes.

Studies examining the electrophysiological properties of cortical regions in AD participants point to some abnormalities in several brain rhythms, as well as in some components of the electrical neuronal signal (Bandelow et al., 2017; Clark et al., 2009; Mueller et al., 2009). Thus, alterations in the slow oscillation rhythm bands have been found throughout the brain, specifically increases in theta band (4-8 Hz) activity and alpha (8-13 Hz) activity, most likely related to biased information processing. Moreover, some abnormalities in beta rhythm (> 13 Hz) oscillations (increases in the band power) have also been observed, but only in frontal and central areas, which are widely linked to interoception, autonomic regulation and reaction to motivationally salient stimuli (see Knyazev, 2012). The frontal regions of OCD participants show excessive EEG power across rhythms, which may be associated with the neurocognitive deficits they present (Kamaradova et al., 2016; Koprivova et al., 2011).

Moreover, an asymmetrical frontal activity between lobes has been found among participants with anxiety disorders (Peltola et al., 2014; Thibodeau, Jorgensen, & Kim,

2006). Although results were not as consistent as expected and a classification focused more on concrete symptoms/signs instead of opaque entities (e.g., a RDoC-related classification) would improve understanding (Adolph & Margraf, 2017; Nusslock et al., 2015), participants high in anxious apprehension (more characteristic in GAD) tend to show elevated relative left frontal alpha activity, while participants high in anxious arousal (more characteristic in panic, social phobia, etc.) show elevated relative right frontal alpha activity. Moreover, Nusslock et al. (2015) has highlighted that participants with higher anxious apprehension and depression may not show the decreased relative left frontal alpha activity that depressive participants often exhibit due to a cancelation effect of anxious symptomatology. This fact constitutes some additional evidence to considering mental disorders in a more flexible way, as put forward by the RDoC initiative (see Insel, 2014).

With regard to EP and ERP, studies point to cognitive biases of anxiety disorder individuals reflecting poor response inhibition (impoverished frontal N2/P3 sequences), biases in information processing (anomalies in N1 component) and abnormal performance monitoring (error-related negativity) across disorders; cortical hyperresponsivity in PTSD (elevated P3, late positive component and contingent negative variation components), and tonic hypervigilance (enlarged P1 components) in social phobia, GAD and specific phobias (for a review, see Bandelow et al., 2017; Clark et al., 2009).

1.3.3. Peripheral Physiological Biomarkers

Peripheral manifestations of anxiety are triggered from top-down mechanisms through three main axes (see Thayer & Sternberg, 2006): the peripheral nervous system (PNS), the HPA axis and the autonomous nervous system (ANS).

The peripheral nervous system allows for sensorimotor control and interaction with the environment (see Buschges, 2005). PNS manifestations of anxiety can be seen

in terms of muscle activity regulation. In this regard, some studies point to elevated muscle tension (especially in the corrugator, a facial muscle involved in the startle reflex commonly seen when confronting fearful stimuli) as a result of experiencing high levels of anxiety or when confronting stressful situations (Kenny, Fortune, & Ackermann, 2011; Smith, Bradley, & Lang, 2005). Moreover, a main feature of GAD and panic is generalized muscle hypertension (Lissek et al., 2010; Pluess, Conrad, & Wilhelm, 2009). In turn, muscle relaxation techniques enable ameliorated muscle tension and improved anxiety symptomatology (see Conrad & Roth, 2007).

The HPA axis is involved in hormonal and immunological regulation in response to context adjustment, especially when facing stressors or challenging events (see Kyrou & Tsigos, 2009; Lupien, McEwen, Gunnar, & Heim, 2009; Ulrich-Lai & Herman, 2009). Stress hormone dynamics (cortisol levels in urine or saliva, adrenocorticotropic hormone, corticotropin releasing hormone, epinephrine and norepinephrine) have been extensively associated with anxiety and anxiety disorders. In this regard, elevated levels of stress hormones (mainly adrenocorticotropic hormone and cortisol) under resting conditions have been observed (Bandelow et al., 2017; Faravelli et al., 2012; Graeff & Zangrossi, 2010; Greaves-Lord et al., 2007). Moreover, an attenuated HPA reactivity has been proposed among participants who exhibited an anxiety disorder when coping with stressors (Gustafsson, Gustafsson, Ivarsson, & Nelson, 2008; Klumbies, Braeuer, Hoyer, & Kirschbaum, 2014; Petrowski, Wintermann, Schaarschmidt, Bornstein, & Kirschbaum, 2013).

With regard to immunological regulation, some hypotheses point to certain inflammatory markers being elevated in anxiety disorders and chronic conditions of anxiety (Salim, Chugh, & Asghar, 2012; Vogelzangs, Beekman, de Jonge, & Penninx, 2013; Weik, Herforth, Kolb-Bachofen, & Deinzer, 2008). Immunological responses are encouraged by stress hormones but mediated by autonomic nervous mechanisms (see Bandelow et al., 2017). Mounting consistent evidence supports an alteration of the immune system in individuals suffering an anxiety disorder. Moreover, some studies have examined the role of some immunological markers (e.g., pro-inflammatory cytokines) that could prolong an inflammatory state possibly leading to hyperactivation

of the sympathetic branch of ANS, as well as hypoactivation of the parasympathetic branch, a very common imbalance found in individuals with anxiety disorders (see Thayer & Lane, 2009; Thayer & Sternberg, 2006).

Finally, the key role of ANS in the expression of (peripheral) physiological signs of anxiety deserves mention. ANS is involved in regulating processes such as heartbeat, breathing, urinary secretion, etc. (Gabella, 2001; Karemaker, 2017). These processes are often altered when an anxiety-related (or a stressful) stimulus is present or high levels of anxiety are experienced. ANS-mediated regulation is done by means of the dynamic interplay between its two branches to preserve the organism's balance (Porges, 2001; Thayer & Sternberg, 2006): the sympathetic branch and the parasympathetic branch. Thus, the sympathetic branch of ANS is responsible for energizing and mobilizing the organism to defensive responses (e.g., accelerating the heart rate to elicit fight-or-flight responses). Conversely, parasympathetic fiber activation leads to restorative responses being initiated (vagally mediated in most instances) to recover baseline or daily states of the organism (e.g., slowing down respiratory and cardiac activity after confronting anxiogenic or stressful stimuli).

Peripheral manifestations of anxiety enervated by ANS are shown across different systems of the organism. In general terms, anxiety is characterized by sympathetic activation and parasympathetic withdrawal (Gulewitsch et al., 2014; Kreibig, 2010; Roth, 2005; Thayer & Lane, 2009; Wilhelm, Trabert, & Roth, 2001). In this sense, either the exposure to anxiety-related conditions or suffering an anxiety disorder are usually related to increases in systolic and diastolic blood pressure, decreased finger pulse amplitude, elevated electrodermal activity, an increased respiratory rate (or an unstable rate in individuals with a panic disorder) in conjunction with decreased end-tidal carbon dioxide concentrations, abdominal pain and stomach upset.

1.3.3.1. Heart-derived Biomarkers for Anxiety

Most physiological processes, as well as cognitive and affective ones, are highly dependent on heart activity due to its key function in mobilizing metabolic resources toward action or restoration (Porges, 2001; Shaffer, McCraty, & Zerr, 2014; Thayer & Lane, 2009; Thayer & Sternberg, 2006). In turn, heartbeat is regulated by ANS branches by means of top-down control mechanisms stemming from prefrontal cortical structures (with the main involvement of the right hemisphere structures). Both branches work synergistically to ensure the heart responds efficiently to adjusting environmental demands.

Different correlates of heart function over time can be studied through various devices monitoring cardiac electrical activity (see Camm et al., 1996, for a review). Electrocardiograms (ECG), pulsometers and heart rate monitors are the most commonly used devices. Traditionally, two sets of heart-derived measures are found to account for cardiac function. The first is more closely linked to time and thus known as the time-domain set of measures. The second is derived from the decomposition of heartbeat fluctuations over time into power of main frequency components. In this way, the so-called frequency-domain cardiac measures are extracted.

In terms of time-domain measures, the most common is heart rate (HR). Heart rate consists of the average number of heartbeats per unit of time (for instance, minutes). HR can be also obtained from calculating the amount of interbeat intervals per unit of time. HR may reflect the relative dominance of the sympathetic and parasympathetic ANS branches on heartbeat (Shaffer et al., 2014). Thus, when an individual is under resting conditions, the parasympathetic branch shows a dominant influence over heartbeat. Increases in HR are shown when the sympathetic branch takes more control over heartbeat as a consequence of contextual demands. Likewise, HR decelerations may be explained by a predominant influence of parasympathetic ANS on heartbeat. Bearing in mind this rationale, individuals with anxiety disorders or high levels of anxiety would show greater HR levels than healthy controls. Some relevant

studies are in this line (see Table 1), providing some evidence on the relative sympathetic dominance observed through HR under resting conditions (Agorastos et al., 2013; Baumert et al., 2009; Bornas et al., 2015; Yeragani, Nadella, Hinze, Yeragani, & Jampala, 2000) as well as when participants are exposed to mood induction tasks (McTeague, Lang, Wangelin, Laplante, & Bradley, 2012; Pittig, Arch, Lam, & Craske, 2013). However, other studies have failed to show how HR may reflect the sympathetic/parasympathetic imbalance in individuals with anxiety disorders or high levels of symptomatology, both under resting conditions (Alvares et al., 2013; Balle, Tortella-Feliu, & Bornas, 2013; Pittig et al., 2013) as well as when exposed to emotion induction tasks (Fisher & Newman, 2013; Gaebler, Daniels, Lamke, Frydrich, & Walter, 2013; Hauschildt, Peters, Moritz, & Jelinek, 2011). These inconsistencies could be related to differences in experimental designs across studies (e.g., length of tachograph) or the influence of associated factors (e.g., gender, prescribed pharmacological treatments, pathological comorbidities, etc.). Nonetheless, it is worth noting that HR may not be as accurate as expected. The HR time series is characterized by beat-to-beat variability over a wide range; in other words, the influence of parasympathetic and sympathetic dominance on heartbeat as a consequence of their competing interplay is too slow to produce beat-to-beat changes, especially given the slow activation of the sympathetic branch (see Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012; Thayer & Lane, 2009).

Some theorists have put forward another, more accurate, biomarker to account for the imbalance between sympathetic and parasympathetic ANS branches in heartbeat characteristics for various disorders (Beauchaine & Thayer, 2015; Friedman, 2007; Thayer & Lane, 2009). In this regard, the alternating dominance of both branches on heartbeat over time would be reflected in response to environmental demands by an oscillatory pattern. The cardiac system of a healthy individual would therefore react to changing environmental conditions by showing some variability to promote better adjustment to them. As a disease is characterized by problematic context adjustment, lower variability (and, subsequently, flexibility) would, for instance, be expected in participants suffering high levels of anxiety symptoms.

Table 1. Cardiac system function in individuals with anxiety: main hypotheses and some relevant evidence.

	Main hypotheses	Studies supporting these hypotheses	Studies rejecting these hypotheses
Resting studies	Higher HR in ANX	Agorastos et al., 2013; Dieleman et al., 2015; El-Sheikh et al., 2011	Alkozei et al., 2015; Blom et al., 2010; Petrowski et al., 2013
	Lower parasympathetic dominance in ANX	Agorastos et al., 2013; Alvares et al., 2013; Balle et al., 2013	Alkozei et al., 2015; Pearson et al., 2005
Reactivity studies *	Lower HR when confronting stressful stimuli in ANX	Klumbies et al., 2014; Petrowski et al., 2017; Pittig et al., 2013; Schmitz et al., 2013	Duncko et al., 2006; Fisher & Newman, 2013; Hauschildt et al., 2011
	Attenuated parasympathetic withdrawal when confronting stressful stimulus in ANX	Bornas et al., 2012; Kramer et al., 2012; Schmitz et al., 2011, 2013	Klumbies et al., 2014; Petrowski et al., 2017
	Attenuated parasympathetic response when confrontation is over in ANX	Alkozei et al., 2015; Kramer et al., 2012; Schmitz et al., 2011	Klumbies et al., 2014; Schmitz et al., 2013

Note. * Reactivity studies displayed in this table refer to studies with stressful stimuli or based on stress induction protocols. HR = Heart rate; ANX = individuals with anxiety (subclinical or clinical syndromes).

Heart rate variability (HRV) is a well-recognized index of the oscillatory behavior of interbeat fluctuations (see Camm et al., 1996; Shaffer et al., 2014). HRV sources are widely used in the context of cardiovascular diseases, cardiac failure and other affections involving the heart. Furthermore, HRV sources may be accurate markers of vagally mediated cardiac regulation of cognitive, affective, and physiological processes. In this sense, HRV tends to be higher when adjustment to context is successful and appropriate emotional regulation is present (Thayer & Lane,

2009). There are varying measures to examine HRV (see Camm et al., 1996): some measures of linear variability fall under the time-domain measures, such as the standard deviation of all NN intervals (SDNN) or the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD); and some fall under frequency-domain measures, such as the power of the very low frequency (VLF) band (.003-.04 Hz), the low frequency (.04-.15 Hz) band power, the power of the high frequency (HF) band (.15-.40 Hz) and the ratio between both powers (LF/HF ratio). HF power (highly linked to respiratory sinus arrhythmia) has been strongly associated with the parasympathetic influence on heartbeat; LF power, despite some controversies still having to be resolved, would index the influence of the sympathetic branch on heartbeat, especially where cardiac activity is monitored over long intervals (e.g., hours or a day); finally, the LF/HF ratio might account for the interplay between both branches on heartbeat. As a key time-domain index, the RMSSD is the most commonly used linear-variability measure to account for the influence of the parasympathetic branch on heartbeat. This measure is highly correlated to HF power but less influenced by respiratory activity than the latter (see Laborde, Mosley, & Thayer, 2017). However, RMSSD as a linear measure dependent on time, where the focus of interest is held in a nonlinear outcome (heartbeat fluctuations), may turn out to be merely an approach for the study of HRV.

As mentioned above, a lower HRV (as a sign of lower flexibility in adjusting to changing environmental demands) is characteristic of individuals suffering high levels of anxiety symptomatology in comparison to healthy controls (Friedman, 2007; Graziano & Derefinko, 2013; Thayer & Lane, 2009). A strong body of evidence supports this fact under resting conditions, when undergoing an emotion induction task or when interventions to ameliorate anxiety symptomatology are delivered (Balle et al., 2013; Bornas et al., 2006; Chalmers, Quintana, Abbott, & Kemp, 2014; Lee, Chao, Yiin, Chiang, & Chao, 2011; Miu, Heilman, & Miclea, 2009; Petrowski, Wichmann, Siepmann, Bornstein, & Siepmann, 2017).

A very useful paradigm for studying HRV under laboratory settings comprises the use of a stress induction task. Stress induction consists of artificially setting up

challenging and stressful situations that encourage the organism to elicit acute stress-related responses, i.e., responses that enable individuals to rapidly adjust to context. As a result, the HPA axis and top-down fear-related responses are activated. Stress induction protocols often comprise laboratory tasks with at least three stages: an initial examination of the baseline state of functioning, under resting conditions, the stress induction stage, and the recovery (or turning-to-basal, daily functioning) stage. HRV changes are observed across stress induction stages in order to encourage better adjustment to paradigm demands (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014; de la Torre-Luque, Diaz-Piedra, & Buela-Casal, 2017; Kirschbaum, Pirke, & Hellhammer, 1993; Thayer et al., 2012).

Thus, a successful adjustment to a stress-induction paradigm involves a higher sympathetic dominance on heartbeat (in comparison to its dominance under baseline conditions) when stress-related responses are elicited, due to its energizing properties for facing stressor demands. Conversely, a higher parasympathetic dominance should be found when the exposure to stress ends and the organism returns to basal function. In other words, lower HRV is expected when faced with the acute stressor, to then increase as stressor confrontation finalizes. Nevertheless, studies with individuals with anxiety disorders or severe anxiety symptomatology have demonstrated some altered flexibility in the sympathetic/parasympathetic control on heartbeat, leading to maladaptive adjustment to acute stressors. In this regard, some studies point to a hypoactivation of the sympathetic branch on heartbeat and heightened parasympathetic vagal reactivity when faced with the stressor (Bornas, Riera, Tortella-Feliu, & Llabres, 2012; Kramer et al., 2012; Schmitz, Tuschen-Caffier, Wilhelm, & Blechert, 2011). These results are not as consistent as expected (see Duncko, Makatsori, Fickova, Selko, & Jezova, 2006; Klumbies et al., 2014) and further research is required to clarify the key aspects of heart-derived responses to acute stressors in individuals suffering from maladaptive anxiety.

1.3.4. New Approaches to Finding Biomarkers for Anxiety

In addition to the tradition in Medicine, Physiology and allied disciplines have looked at diseases and disorders as deviations from the normative patterns in the regular function of a system, or even in the array of its structural elements. Thus, physiological research has, by and large, focused on identifying the extent of the deviation or variability from normative patterns, by comparing the functioning of relevant systems in a clinical group (presumably, the group with the abnormal, deviated pattern) and a control group of healthy subjects. Mathematically-derived approaches based on linear models are the basis of such rationale. A clear example in this regard can be found in exploring how different cardiac systems function in people with anxiety disorders are in comparison to matched controls. In short, the larger the deviation, the more diseased or disordered the system (for instance, an individual with an anxiety disorder who showed higher levels of HR would suffer from more severe symptomatology, so the heartbeat would reflect how affected this physiological system was).

A little later, a new perspective emerged to address how physiological systems might better adjust to contextual demands: the concept of HRV and how healthy it is to show greater variability across contexts became crucial in examining the physiological systems of disordered people.

More recently, frameworks emerging from Physics and Pure Sciences have encouraged a revolutionary view of variability in the field of Health Sciences. In this regard, concepts such as irregularity or complexity are more appropriate. In the field of Physics, the Dynamical Systems Theory (DST; see Gleick, 1987; West, 2006) allowed some properties of living physiological systems to be studied, such as reproducibility or self-similarity (also called fractal nature) across timescales or the existence of a related built-in long-term memory process involved in their functioning. Hence, these systems need to operate at multiple timescales in order to adjust to environmental demands, since responding solely in a single real-time scale does not suffice. Even at rest, these systems do not remain stable, i.e., they do not fluctuate under a single timescale but

show scale invariance: statistically similar patterns of fluctuations exist at multiple timescales (see, for instance, Bornas, 2016, p. 21-43). Bearing in mind this framework, several groundbreaking discoveries have been made. Many studies have endorsed the nonlinear and complex nature of the emotional subjective system, cardiac system, respiratory system, or human gait (Fiol-Veny, de la Torre-Luque, Balle, & Bornas, 2017; Hollenstein, Lichtwarck-Aschoff, & Potworowski, 2013; Ivanov, Chen, Hu, & Stanley, 2004; Katerndahl, 2009; West, 2006). With regard to the central nervous system, Freeman has shed light on the complex nature of brain dynamics at several timescales (Freeman, 1999). Likewise, a state of self-organized criticality seems to be the brain's default mode of functioning (Chialvo, 2010; Palva et al., 2013; Werner, 2010).

As a consequence, complex, multiscale variability started to be associated with health since a human system may be conditioned by various set points of influence, both intrinsic and extrinsic. Deviations or fluctuations in their functioning over time would therefore be inferred as a result of the competing process among influences. Conversely, non-variable, rigid function of a system across scales has become a pathological biomarker, often linked to the presence of an attractor (e.g., a disease) highly impacting system function, and hindering normative, variable behavior over time. Some authors endorse the hypothesis that a decrease in variability (irregularity or complexity) is often seen as a result of a disease or aging (see Goldberger et al., 2002; Sturmberg, Bennet, Picard, & Seeley, 2015).

Measures derived from the DST perspective (so-called nonlinear measures) have been used in the search for disease biomarkers. Thus, for instance, some nonlinear biomarkers have been identified across physiological systems as a sign of varying medical conditions (Hausdorff et al., 1997; Ho et al., 1997; King et al., 2010; Tapanainen et al., 2002). An extension to mental disorders is derived from this framework aimed at identifying nonlinear biomarkers for anxiety disorders. In this regard, some studies have been conducted in this field but results are not as consistent as expected (see, for instance, Agorastos et al., 2013; Caldirola, Bellodi, Caumo, Migliarese, & Perna, 2004; Chae et al., 2004; Moon, Lee, Kim, & Hwang, 2013). Wider

research needs to be carried out in order to ascertain which factors determine these divergences across studies.

More currently, some studies from the DST perspective point to an excessive variability possibly being a signal of a disease or disorder, for instance when exploring the respiratory fluctuations of panic-disorder individuals or the hypothalamus-pituitary-adrenals axis released in depressive patients (Caldirola et al., 2004; Carroll et al., 2012). As a consequence, two theoretical postulates have emerged to integrate these findings with the key assumption of higher complexity as a sign of health: Yang and Tsai (2013) have suggested that some pathological entities are derived from an absence of variability or complexity (e.g., cardiac function of anxiety disordered individuals) whereas an excess of variability (or a random-like variability) would also lead to the pathological function of a physiological system (e.g., the blood-oxygen intake in orbitofrontal, occipital, and postcentral brain areas among schizophrenic patients). In turn, Schuldberg (2015) has introduced the concept of optimum variability, defined as the most desirable level of variability in the direction of system growth or movement over time. Thus, the degree of variability in a system must be optimal depending on how the system maximizes adaptive benefits and reduces costs.

In short, applications of DST to explain physiological processes are contributing to a consistent background to better understand how maladaptive anxiety may alter the functioning of an individual's physiological system. However, further research should be conducted to obtain valuable biomarkers for diagnosis and intervention.

1.4. Developmental Psychopathology: Adolescence as a Critical Period for the Expression of Maladaptive Anxiety

1.4.1. Normative Development of Anxiety in Adolescence

Adolescence is a crucial period in life running from age 10 to 21. Neinstein's traditional and renowned characterization of the different stages of adolescence are (see Radzik, Sherer, & Neinstein, 2008): early adolescence (approximately ages 10-13), middle adolescence (14 to 16) and late adolescence (17 to 21). In terms of a transition hypothesis, maturational development is the essential process throughout adolescence whose effects lead to changes in physiological and emotional systems towards adulthood. For this reason, adolescence has traditionally been considered a turbulent period in life and, in this regard, G. Stanly Hall's "storm and stress" hypothesis from the early 20th century is well known in the study of adolescence and development (Hall, 1904). Hall stated that individuals throughout adolescence may show significant decreases in self-control (storm) and increases in sensitivity to arousing stimuli (stress) as a consequence of biological, maturational processes (e.g., the reorganization of some brain areas, the release of sexual hormones, etc.) in comparison with other periods in life. Some evidence from key domains in adolescent life endorse the storm and stress hypothesis (see Hollenstein & Lougheed, 2013): conflict with parents (more frequently in early adolescence but with the highest intensity in mid adolescence), mood disruptions (more common in mid adolescence), and risk taking behavior (more likely in late adolescence).

More recently, new approaches have put forward dynamical interactions between physiological systems, maturational readiness and environmental demands on the organism and put into question how accurate the storm and stress hypothesis of development is across adolescence (Graber, Brooks-Gunn, & Peterson, 1996; Granic, 2005). Hollenstein and Lougheed (2013) have proposed the so-called 4T Model to account for adolescence as a period in life and the relative impact of maturation on it.

These authors have stated that adolescence involves an integration of four key aspects: typicality (maturational changes are typical in this period), temperament (as a constraint of biologically determined emotional and behavioral responses), transactions (understood as the dynamic interactions between maturing physiological processes and contextual factors) and timing (timing needs a maturational change to be completely finalized). Taking into consideration these assumptions, the authors aimed to highlight how inevitable maturation across adolescence is (biological readiness). Nonetheless, based on how these maturational changes are confronted, adolescents may develop behavioral repertoires (e.g., some types of emotion regulation strategies) that modulate certain aspects of maturation, such as the onset of specific changes or their duration, or the intensity by which maturation manifests. Furthermore, contextual conditions may alter maturational processes (e.g., exposure to recurrent high stress levels).

When it comes to emotional aspects, substantially novel patterns of coping with stress, changes in reactivity to different emotional stimuli, and new strategies of emotion regulation may often arise in adolescence in comparison to earlier life periods (Eiland & Romeo, 2013; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Hollenstein & Lougheed, 2013; Skinner & Zimmer-Gembeck, 2007). One supporting factor for this theory is adolescents possibly evaluating events as more stressful compared to children as they tend to experience more events in a more stressful manner (Hampel & Petermann, 2006; Wiklund, Malmgren-Olsson, Ohman, Bergstrom, & Fjellman-Wiklund, 2012).

Adolescence is a paramount period for the developmental course of anxiety. Weems (2008) has, from a longitudinal viewpoint, proposed that maladaptive anxious emotion may lead to elevated levels of symptomatology or even the development of an anxiety disorder as a consequence of the dynamic interaction between individual and contextual factors, and taking into account its basal natural course.

Studies on developmental psychopathology have aimed at depicting how anxious symptomatology evolves over adolescence. Accordingly, some studies have highlighted a pattern of smoothly curvilinear decrease of overall anxiety (Bongers, Koot, van der Ende, & Verhulst, 2003; McLaughlin & King, 2015; van Oort et al.,

2011), whereas others have set out a more linear, even increasing, pattern (Betts et al., 2016; Leadbeater, Thompson, & Gruppuso, 2012). These discrepancies could be due to the different lengths of the studied periods (usually entire adolescence, with some even including long periods of childhood or adulthood), as well as to the assessment timing (annually or biannually). Moreover, there is no a consensus as to the stability of experiencing anxiety symptomatology over time, with existing studies showing between 4-80% symptomatology stability (see Weems, 2008). Quoting Weems:

"These studies may show wide variability for many reasons (e.g., the type of disorder, the informant, the sample, the method of assessment, or the amount of time that has passed between the initial evaluation and the follow-up). Interestingly, however, studies have shown similarly wide estimates even for the same anxiety disorder across similar time frames using similar methodology." (Weems, 2008, p.490)

An RDoC-based approach could probably resolve these discrepancies between studies since a categorization of anxiety-related conditions is not required when considering different levels of analysis of the same complex condition.

In turn, two important issues should be taken into consideration. Firstly, adolescence constitutes a sensitive period for the development of anxiety disorders (see Paus, Keshavan, & Giedd, 2008). However, not all anxiety disorder syndromes are expressed within adolescence, or not with the same severity. For this reason, it should be said that (early and mid) adolescence is a sensitive period for some anxiety disorder subtypes. According to Weems (2008), differential patterns of anxiety expression can be seen as adolescence progresses. Thus, separation anxiety symptoms and animal phobias are commonly experienced in childhood but tend to fade away in early adolescence. A predominance of GAD symptomatology expression may be reported more throughout youth and early adulthood (Copeland et al., 2014; Seligman & Gahr, 2013). Social phobia symptomatology is predominant from early to middle adolescence, with this

period showing the highest prevalence rates of full-blown social phobia diagnoses in comparison to any other (Beesdo-Baum & Knappe, 2012; Kessler et al., 2012).

The second key concept in the course of anxiety is the continuity of symptoms. Weems (2008) has proposed a sequential course of different ADs, in the sense that some of them would precede others (a heterotypic course of anxiety). Thus, for instance, separation anxiety would be an antecedent of overanxious disorder, or exam anxiety would precede generalized social phobia. Conversely, and as mentioned in a previous chapter, homotypic continuity, or how the presence of a specific type of anxiety symptomatology may predict experiencing a subsequent onset of a similar syndrome, should be taken into account. In this regard, research has shown some mixed evidence partially favoring both concepts (see Asselmann & Beesdo-Baum, 2015; Zavaglia & Bergeron, 2017). Ferdinand et al. (2007) studied how the presence of high symptomatology of specific anxiety disorders at age 10-12 may predict high levels of symptoms from a similar disorder or others two years later. As a result, they observed large homotypic continuity in terms of panic and social phobia symptomatology, higher predominance of heterotypic continuity for OCD symptoms, and a roughly similar influence of heterotypic and homotypic symptoms for GAD and separation anxiety. More recently, Snyder, Young, and Hankin (2017) have assessed anxiety symptomatology in adolescents at two measurement points (T2 was 18 months after T1). They found strong support for homotypic continuity, reporting a between-assessment correlation of .71. Ormel et al. (2015) have also found high homotypic continuity among anxiety disorders in adolescence, suggesting that anxiety disorders may be chronic disorders with episodes of higher and lower symptomatology throughout life. These authors, however, have also highlighted a moderate influence of other disorders on anxiety, pointing to a clear role for preexisting moods or drug abuse disorders on the development of an anxiety disorder.

1.4.2. Temperament Traits as Risk Factors for Maladaptive Anxiety in Adolescence

Overall risk factors for anxiety disorders have been addressed in chapter 1.2.3. However, it is worth looking further at one of them—temperament—due to its relevance in the manifestation of significant levels of anxiety across adolescence. Risk factors such as gender and socioeconomic status have therefore been disregarded in this chapter. Likewise, parental and personal history of diseases or mental disorders have not been considered either. The main reason for this is that these factors may modulate the readiness to manifest maladaptive anxiety in a relatively similar way to other periods in life (Hirshfeld-Becker et al., 2008; Moreno-Peral et al., 2014). Contextual risk factors are also vivid for the development of adolescent anxiety disorders, particularly traumatic or stressful events (Gulley, Hankin, & Young, 2016; Laceulle, Nederhof, Karreman, Ormel, & van Aken, 2012). However, these events are not usual and universal for all cultures, geographic regions or populations, i.e., they would not necessarily be experienced by adolescents on a daily basis and, therefore, are not necessarily involved in the development of anxiety throughout adolescence.

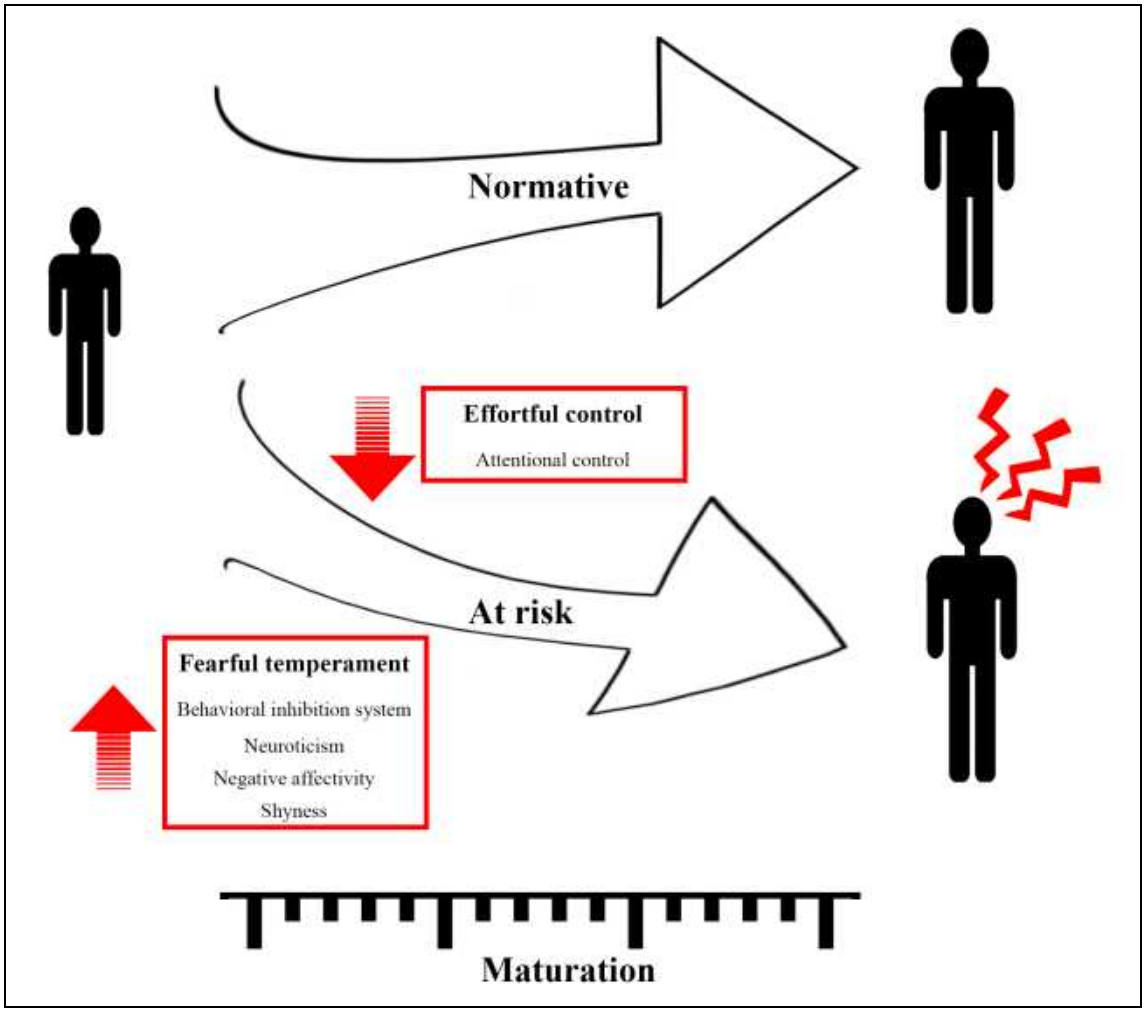
As the precursor to adult personality, temperament is broad and general behavior-influencing trends with a major inherited component, albeit modulated by environmental demands, to reach contextual adjustment (Nigg, 2006; Rothbart, 2007; Soto & Tackett, 2015). Several temperamental traits may place adolescent individuals at risk of developing high anxiety (see Figure 2). It is important here to highlight the impact of so-called fearful temperament (FT) in the expression of maladaptive anxious emotion (see Rapee & Coplan, 2010). Under FT, diverse traits may be embraced according to different theoretical frameworks. All these traits as components of reactive temperament make individuals feel defensive emotions (e.g., fear) to novelty or unfamiliar situations and avoid potentially threatening stimuli. More specifically, constructs related to the major behavioral inhibition system (BIS) from Gray's Reinforcement Sensitivity Theory are defined as FT traits (Bijttebier et al., 2009; Gray, 1982; Gray & McNaughton, 2000). Consequently, BIS is responsible for organizing

behavioral responses toward inhibition and avoidance. BIS-related responses are mediated by the fight-flight-freeze system (FFFS). Some studies have pointed to higher BIS and FFFS scores (historically and concurrent to anxiety assessment) in anxious children and adolescents (although with no differences in the behavioral approach system—the other major temperamental component from Gray's theory) always in interaction with other factors, such as an adolescent's history of mental problems and other temperament factors (Bornas et al., 2015; Rapee, 2014; Sportel, Nauta, de Hullu, de Jong, & Hartman, 2011; Vervoort et al., 2010). Additionally, some well-known alterations in emotional information processing of individuals with anxiety disorders (e.g., alterations in face processing and attention biases to threat) have been linked with higher BIS scores (Perez-Edgar et al., 2010; Reeb-Sutherland et al., 2009, 2015).

Neuroticism, a traditional FT trait, should be underlined. In this regard, neuroticism refers to the tendency to experience negative emotions or unpleasant affects. Rothbart's framework construct related to neuroticism, the negative affectivity (NA) trait, has been extensively studied in adolescence (Capaldi & Rothbart, 1992; Rothbart, 2007). Similar to BIS, NA has been strongly linked to high anxious symptomatology and the development of ADs (Bosquet & Egeland, 2006; de Pauw & Mervielde, 2010; Gulley et al., 2016; Muris, de Jong, & Engelen, 2004; Tortella-Feliu, Balle, & Sese, 2010).

Furthermore, another temperament trait highly related to social issues should be mentioned: shyness. This trait is defined as the tendency to experience a state of discomfort in social situations and to escape them if possible (Henderson, Gilbert, & Zimbardo, 2014). Despite shyness not necessarily involving anxious emotion, it has also been associated with anxious symptomatology, particularly social phobia (Rubin, Copland, Bowker, & Menzer, 2011; Tsui, Lahat, & Schmidt, 2016). Children with higher increases in shyness across mid-to-late childhood tend to exhibit elevated anxiety in early adolescence, and they are likely to maintain heightened anxious levels throughout adolescence (Karevold, Ystrom, Copland, Sanson, & Mathiesen, 2012).

Figure 2. Temperamental factors that put adolescents at risk of high maladaptive anxiety. Red rows refer to directionality of said factor.



In turn, a component of regulatory temperament should be noted: effortful control. EC comprises the ability to focus attention away from salient stimuli, to inhibit a dominant behavioral response and encourage another more contextually relevant one (Rothbart, 2007). Low EC has been clearly linked to significantly high anxiety symptomatology and the development of ADs, as well as to the objective correlates of typical alterations in emotional processing among anxiety-disorder individuals (Bornas et al., 2015; Gulley et al., 2016; Lonigan & Vasey, 2009; van Oort et al., 2011; Wauthia & Rossignol, 2016). One EC component is especially relevant: attentional control (AC);

this is the ability to voluntarily modulate attentional resources from a dominant response to a subdominant one that is more adaptive in a specific context. Low levels of AC have also been linked with anxiety symptomatology and the development of anxiety disorders in adolescents (Muris et al., 2004; Sportel et al., 2011).

1.4.3. Cardiac Function in Adolescents with Maladaptive Anxiety

Adolescence constitutes a paramount period for the maturation of physiological systems. In terms of cardiovascular physiology, morphological and functional changes occur gradually from early life years to around mid-adolescence, when an adult-like pattern of cardiac regulation is shown (see Chan, Sharieff, & Brady, 2008; O'Connor, McDaniel, & Brady, 2008). Thus, a higher dominance of the ANS parasympathetic branch on the beating heart at rest is observed over time and, therefore, lower HR and higher parasympathetic-related indices of HRV are found in adolescents in comparison to children (Eyre, Duncan, Birch, & Fisher, 2014; Fleming et al., 2011; Kowalewski, Alifier, Bochen, & Urban, 2007). This may be related to maturational mechanisms (e.g., maturation of prefrontal brain areas and GABAergic circuitry) that enable more efficient brain-viscera integration and a higher influence of top-down inhibitory control on physiological processes (Luna, Marek, Larsen, Tervo-Clemmens, & Chahal, 2015; Thayer & Lane, 2009).

Adolescent cardiovascular activity is influenced by many factors: psychopathological features may be involved in the regulation of heart function as observed in other periods in life (see, for instance, Beauchaine & Thayer, 2015; Friedman, 2007; Graziano & Derefinko, 2013). More concretely, anxiety symptomatology is related to deviations from normative patterns of heart function under resting conditions, as well as under conditions of reactivity (phasic conditions). Mounting evidence supports the hypothesis of a dysregulation in heart adjustment to daily conditions when a maladaptive anxiety condition (high levels of anxiety symptomatology or an AD) is experienced. Thus, the attenuated vagal control on

heartbeat and the subsequent heightened dominance of the sympathetic branch are usually observed in adolescents with anxiety disorders under resting conditions (Dieleman et al., 2015; Schmitz et al., 2011). Nonetheless, certain other factors should be taken into account, such as gender (parasympathetic-related markers may better reflect this cardiac dysregulation in girls compared to boys) or age (Blom, Olssom, Serlachius, Ericson, & Ingvar, 2010; El-Sheikh, Arsiwalla, Hinnant, & Erath, 2011).

Some controversy, however, is seen in reference to how accurate some heart-derived biomarkers are among at-risk adolescents. In this regard, some studies have endorsed the greater parasympathetic withdrawal baseline or the heightened sympathetic influence of heartbeat as biomarkers for anxiety disorder risk in adolescents (Balle et al., 2013; Schmitz, Tuschen-Caffier, Wilhelm, & Blechert, 2013; Scott & Weems, 2014), whereas others have not (McLaughlin, Alves, & Sheridan, 2014; Viana et al., 2017).

A more valuable contribution in the search for cardiac biomarkers of anxiety disorder risk may come from another (non-static or baseline-based) perspective. In this sense, mounting evidence supports a loss of adolescent cardiac flexibility in adjustment to challenging tasks requiring the dynamic interplay of sympathetic and parasympathetic ANS branches on heartbeat. Therefore, and as stated in a previous chapter, a sympathetic dominance on heartbeat and a subsequent parasympathetic withdrawal when undergoing stressful tasks would be expected. Studies in at-risk and full-blown anxiety-disorder adolescents have shown a blunted increase in sympathetic activity as well as a lower parasympathetic withdrawal when undertaking the challenging, stressful task and afterwards (Graziano & Derefinko, 2013; Kramer et al., 2012; Schmitz et al., 2013). Perhaps the most supported hypothesis postulated in this vein is a reduced activation of the energizing physiological mechanisms (the HPA axis and sympathetic branch influence on heartbeat) when confronting stressors, as a result of their overreactivity to different stimuli deemed stressful by individuals with high levels of anxiety (Chida & Hamer, 2008; Klumbies et al., 2014; Siess, Blechert, & Schmitz, 2013). As a consequence, this may lead to an exhaustion of the related physiological systems (see McEwen, 2006 for a review). More research should be

carried out to disentangle how this mechanism could fall under dysregulation from subclinical to full-blown clinical anxiety disorders in adolescents.

1.4.4. New Approaches to Studying Anxiety in Adolescence

Nowadays, science studies increasingly complex phenomena that require interdisciplinary approaches and studying adolescent anxiety is not an exception. Thus, the approach for adolescent anxiety involves a major effort to gain support from different areas of study, such as developmental research and psychopathology. However, a large amount of studies done thus far have focused on drawing a static picture of how psychopathological features (and anxiety) may enable us to distinguish between "healthy" adolescents and adolescents with elevated mental distress, both in the throes of maturation (see, Cicchetti, 1993). Likewise, the dynamical influence of different factors on psychopathological features (it would be logical to think that if a risk factor changes over time, its influence on a concrete condition will vary as well) is often overlooked (see, for instance, Betts et al., 2016; Olino, Klein, Lewinsohn, Rohde, & Seeley, 2010) leading to misleading conclusions and inaccurate (non-replicable across age groups) findings.

According to the classical premise in developmental psychology ("past behavior is the most reliable predictor of future behavior"), research in the field of developmental psychopathology should involve taking a longitudinal perspective analyzing relevant behaviors. In this regard, it is crucial to consider well-established starting points and periods of assessment in order to elucidate how anxiety evolves over time. Therefore, a major effort should be made to accurately determine assessment timing and intervals between assessments. For instance, and as stated in an earlier chapter, adolescents are often assessed annually or biannually. However, maturational processes may work at relatively shorter timescales and their interaction with contextual factors may lead to drastic changes in emotion regulation and daily adjustment (Bornas, Llabres, Balle, de la Torre-Luque, & Fiol-Veny, 2014; Vanderhasselt, Brose, Koster, & De Raedt, 2016).

Therefore, relevant developmental changes may go unnoticed if the consecutive assessment points are too far apart.

In turn, person-centered factors or how individuals confront and perceive daily and unexpected events in life should be taken into consideration when depicting how anxiety evolves across adolescence (Weems, 2008, p. 494). These aspects have scarcely been addressed thus far. Recently, an interest in depicting the different heterogeneous developmental courses of anxiety symptomatology has emerged (see, for instance, Morin et al., 2011; Nelemans et al., 2014). Fortunately, new methodological and data analysis frameworks have already enabled flexibly modelling for individual-related courses over time within the general trend and the possibility of dealing with the dynamical influence of covariates over time (Dantan, Proust-Lima, Letenneur, & Jacqmin-Gadda, 2008; Proust-Lima, Sene, Taylor, & Jacqmin-Gadda, 2014).

From a more experimental perspective, many researchers and health professionals complain about how far findings obtained under laboratory settings are from everyday life. For instance, physiological cardiac research findings from many studies come from laboratory settings and short monitoring. Of course, these findings are highly valuable due to the controllability of laboratory protocols. However, their ecological validity and subsequent generalizability can be questioned (see Frick, Barry, & Kamphaus, 2010; Vaitl & Schandry, 1995). Adolescents do not live in laboratories or within controlled environments, and the combined influence of multiple factors may influence them on a daily basis. For this reason, a more ecological point of view to conducting research should be considered today. Bornas et al. (2015) assessed cardiac dynamics on a regular class day for adolescents at risk of anxiety disorders in comparison with adolescents with low anxiety symptoms. As a result, they observed a significant between-group discriminative power of some nonlinear cardiac measures (fractal dimension and multiscale entropy). However, linear measures related to parasympathetic functioning failed to differentiate between groups under this realistic context. More research is therefore needed to clarify the cardiac function patterns (or other physiological or subjective systems) under more realistic conditions.

Finally, and in line with the DST framework, some authors have made major efforts in understanding developmental processes dynamically over time. Granic (2005) has described in an outstanding theoretical paper how developmental researchers may draw on the varying DST tools to examine psychopathology in childhood and adolescence (this could also be applied to any other period in life). Thus, the author has stated that developmental changes are gradually affecting socioemotional and physiological processes across different timescales (moment-to-moment, day-to-day, month-to-month, etc.). Further, variability in a human system in development may be part of its regular multistability pattern of activity (it does not operate at a single timescale, but at multiple ones). Only with the presence of an attractor could system variability become highly predictable. Attractors can be defined as follow:

"Attractors are stable patterns of interactions or absorbing states that "attract" or pull the system from other potential states. Behavior moves toward these attractors in real time and, to the extent that this movement is indeterminate, this can be described as self-organization at the scale of real time." (Granic, 2005, p. 392)

Anxiety conditions may show attractor-related patterns that emerge over days, months or years when studying several human systems (see Bornas, 2016; Granic, 2005).

More recently, Hollenstein et al. (2013) have proposed the so-called Flex3 model to account for variability in the human socioemotional system. They have postulated that flexibility is a key feature of human systems and characterized by variability over time and across different, interacting timescales. They have put forward the existence of three main timescales: micro, meso and macro. The micro scale accounts for variability within a context or specific situation. Within this scale, cardiac variability when confronting a stressful task, for instance, is addressed. Through the meso scale lens, rigidity or flexibility would be noted across contexts. Thus, changes in emotional regulation, psychopathological symptomatology (e.g., changes due to

delivering an intervention protocol) and relations with others would be studied with this scale. Finally, the macro scale perceives variability developmentally or as a trait. Variability in temperament, personality or other stable constructs would be understood within this scale with changes manifested at monthly or yearly timescales. Additionally, the Flex3 model postulates some dynamic interaction between the variability patterns across timescales. Therefore, interactions between temperament (macro) and psychopathology (meso), or between physiological adjustment to concrete tasks (micro) and general mood (meso), are assumed.

1.5. Preventing the Development of Anxiety Disorders in Adolescence

1.5.1. Psychological Interventions to Prevent the Escalation of Adolescent Anxiety

A great amount of public and private resources is invested annually to prevent people from developing medical diseases and psychological disorders. Adolescent people often are the target population for government prevention actions in terms of health and welfare policy. For instance, the WHO promotes several initiatives worldwide to prevent illegal substance abuse disorders (see WHO, 2011); also the National Institute of Mental Health of the United States encourages and funds preventive interventions devoted to easing emotional symptomatology in adolescence (see McLaughlin, 2011).

A preventive program in mental health sciences aims at targeting specific and non-specific risk factors for concrete mental disorders, enhancing protective factors or reducing symptomatology related to a full-blown disorder and with high associated distress (Gladstone, Beardslee, & O'Connor, 2011). Prevention efforts may help to reduce the social and economic burdens of mental disorders and to relieve the mental distress associated with them (WHO, 2004, p. 15).

Various tools developed under psychological framework principles can be used for preventive purposes. Psychological preventive programs can be classified according to the target population (Mrazek & Haggerty, 1994). Thus, universal preventive programs constitute the first and most general type. Universal programs target prevention efforts among the general public, i.e., all individuals from a community receive these interventions regardless of whether they present one or more risks and/or protective factors. In turn, there are targeted interventions aimed at either tackling some risk factors in developing a specific disorder or encouraging protective factors. There are two types of targeted programs: the selective preventive programs targeting populations and those targeting individuals with elevated levels of specific and non-specific risk factors for the development of a disorder or lower levels of protective risk factors. In the context of adolescent ADs, selective preventive interventions may be delivered to reduce the influence of some risk factors (e.g., anxiety sensitivity or poverty) or to enhance some protective factors (e.g., problem-solving skills) in school-based but also community contexts (Balle & Tortella-Feliu, 2010; Gillham et al., 2006; Kindt et al., 2014). In preventive interventions focused on individuals with high (subclinical) levels of symptoms, there are many interventions for adolescent anxiety disorders aimed at ameliorating anxiety symptomatology in at-risk individuals (see, for instance, Manassis et al., 2010; McCarty, Violette, Duong, Cruz, & McCauley, 2013).

In recent years, mounting studies have tested the effectiveness of prevention programs to reduce anxiety symptomatology and the influence of risk factors for the development of anxiety disorders. Nevertheless, their findings are highly contradictory and some meta-analytic studies have been needed to form an integrative picture (Ahlen, Lenhard, & Ghaderi, 2015; Stockings et al., 2016). The results from meta-analytic synthesis are not as promising as expected, with a null to low effect size for prevention programs being observed in comparison to waiting list and placebo conditions. Stockings et al. (2016) have found a low effect size for prevention programs (even when targeted interventions were only considered) with significant reductions of anxiety up to three months after delivering the interventions. The authors have indicated that preventive programs should include active educational and psychological components to attain higher effects in terms of anxiety reduction.

Interventions delivered within school contexts seem to be more effective. Some of the advantages of these so-called school-based programs may be related to implementation: a) the programs are delivered within a familiar context; b) adolescents can access easily the treatment source; c) school constitutes a context to put into practice the skills adolescents may acquire as a consequence of the intervention delivery. Werner-Seidler, Perry, Calear, Newby and Christensen (2017) have recently published a meta-analytic study to analyze the effects of school-based preventive programs on reducing internalizing symptomatology. As a result, they have found that 24 studies examined the effect of prevention intervention on anxiety symptomatology. A low effect size (g = 0.20) was estimated from these studies. They highlighted that the prevention effects of these interventions were significant up to six months after delivering them. Moreover, there were no differences in the intervention effect sizes in terms of the type of preventive program (universal or targeted). Another important issue was that the effect size derived from implementing the prevention program is related to the program's implementers: they observed a higher effect size for the prevention programs delivered by external staff in comparison to those delivered by school staff. Finally, the authors highlighted the general absence of methodological controls (e.g., random allocation) of the studies to ensure the accuracy of the reported findings.

1.5.2. Transdiagnostic Interventions to Prevent Adolescent Anxiety

Researchers and clinicians complain about how difficult is to find "pure" psychological disorders in clinical and community settings. Individuals attending mental health services often present complex sets of symptoms whose categorization requires exploring several mental health entities to make an accurate diagnosis of the ongoing pathological processes. The RDoC does embrace these complex manifestations, taking into account the fact that several brain and physiological substrates and their interactions may be involved in the symptomatology observed (see Etkin & Cuthbert, 2014).

Emotional disorders (depression and anxiety disorders) are not exempt from this overlapping manifestation. For this reason, many authors and theorists make major efforts to change the standpoint of the scientific community so as to progress toward a unified consideration. David Barlow is one of the leading researchers and theorists encouraging the development of a unified protocol of treatment for emotional disorders. In fact, he and his colleagues have proposed the so-called negative affect syndrome as a unified syndrome covering various anxiety- and mood-related symptoms, due to the commonalities observed between depression and ADs (Barlow, Allen, & Choate, 2004). Many arguments support how much these disorders overlap. Firstly, it is worth mentioning the high comorbidity shown between anxiety and depression, with comorbidity rates close to 70% in some cases, such as the common joint presentation of GAD and a major depressive disorder (Merikangas et al., 2007, 2010). Along the same lines, varying anxiety disorders and depressive syndromes are characterized by similar psychopathological manifestations (e.g., worry and rumination, fatigue, etc.). It is also very common to see an anxiety disorder emerging from a depressive syndrome or vice versa (Balasz et al., 2013; Karsten et al., 2011; Ormel et al., 2015). Moreover, some common risk factors (e.g., neuroticism, poverty, genetic polymorphisms, etc.) may be related with the development of both an anxiety disorder and a depressive disorder in tandem (Blanco et al., 2014; Luciano et al., 2010).

Furthermore, and shifting the focus away from clinical entities, correlations between depressive symptom scores and anxiety-related symptoms often are significantly high (Gotham, Brunwasser, & Lord, 2015; Gulley et al., 2016). This could be related to the latent structure of symptomatology from both types of disorders. In this sense, structural equation modelling studies have shown a strong association between symptoms from both entities and a potential overlap, when models are estimated freely, without prior conceptual constraints (de Carvalho et al., 2014; Liu, Shono, & Kitamura, 2009; Tortella-Feliu et al., 2010).

Finally, several neural and physiological mechanisms are involved in the pathophysiological and external manifestations of both types of disorders. For instance, the role of the amygdala and the orbitofrontal-basal ganglia circuit in the manifestation

of depressive and anxious symptoms is clear (Bandelow et al., 2016; Croarkin et al., 2011), in addition to asymmetrical frontal activity in terms of brain electrophysiology (Nusslock et al., 2015). Further, the HPA is also involved in the manifested signs of anxiety and depression, as well as the higher withdrawal of the parasympathetic branch on the beating heart at rest (Beauchaine & Thayer, 2015; Faravelli et al., 2012; Graziano & Derefinko, 2013).

Bearing in mind these arguments, transdiagnostic interventions have been developed to ameliorate anxiety and depressive symptomatology in clinical samples, as well as in individuals with some risk of developing related full-blown disorders (Clark, 2009; Craske, 2012; Farchione et al., 2012). Transdiagnostic interventions may well retain the advantages observed in diagnosis-specific treatments but with additional benefits, such as the prevention of a new emotional disorder developing (Barlow et al., 2004; McEvoy, Nathan, & Norton, 2009; Norton & Barrera, 2012)

The implementation of emotional-disorder transdiagnostic interventions for preventive purposes has also been considered (Dozois, Seeds, & Collins, 2009; Garcia-Escalera, Chorot, Valiente, Reales & Sandin, 2016). As stated above, many common risk factors have been identified for anxiety and depressive disorders. Thus, delivering interventions to deal with these factors may be useful in tackling the escalation of symptomatology and the development of emotional disorders. Transdiagnostic preventive programs can be applied both universally and as targeted interventions for individuals with at-risk profiles. The results from these applications have been promising although modest, showing low-to-medium effect sizes in terms of anxiety symptomatology reduction up to the 6-month follow-ups (Bilek & Ehrenreich-May, 2012; Queen, Barlow, & Ehrenreich-May, 2014; Topper, Emmelkamp, Watkins, & Ehring, 2017). In any event, transdiagnostic preventive programs seem to show better results when used with adolescents at risk of anxiety disorders. Nevertheless, Garcia-Escalera et al. (2016) have stated that studies testing the effectiveness of these interventions have shown a concerning lack of methodological controls (e.g., a lack of random allocation or meaningful levels of attrition bias) which may put the observed findings into question.

1.5.3. The SUPER-Ad Program

In 2013, Dr. Cecilia A. Essau and Dr. Thomas H. Ollendick published the Super Skills for Life (SSL) program (Essau & Ollendick, 2013). The SSL is a transdiagnostic program aimed at ameliorating internalizing symptoms in children with at-risk profiles. A year later, a study provided some evidence on the effectiveness of delivering this intervention among children, who were referred by their teachers, suffering from anxiety problems (Essau, Lewinsohn, Olaya, & Seeley, 2014).

Researchers from the Center for Applied Research and Assessment in Child and Adolescent Well-being (CARACAW) led by Dr. Essau make major efforts in adapting this program to different at-risk populations (adolescents, young people, etc.) and cultures. Thanks to a collaboration between the CARACAW researchers and members of the Cognitive-Affective Neuroscience and Clinical Psychology (CANCLIP) research group, a Spanish version of the SSL program for adolescents was designed. This program is called SUPER-Ad (de la Torre-Luque et al., 2015).

The SUPER-Ad is a transdiagnostic program designed for prevention purposes and focused on ameliorating internalizing symptoms (anxiety and mood-related symptoms) in adolescents. As the program was written in Spanish, it is designed for and adapted to Spanish adolescents between the ages of 11 and 16. The main aim of the SUPER-Ad program is twofold: (1) to tackle some common risk factors in the development of internalizing disorders (low self-esteem, poor social skills or behavioral avoidance highly related to sensitivity to the punishment temperamental trait), and (2) to strengthen protective factors by implementing different content modules (e.g., problem-solving skills, introducing healthy life patterns). The program was designed in line with cognitive behavior therapy principles and incorporates some behavioral activation components. It can be delivered on community basis but also as a school-based intervention (delivered within school settings). It comprises eight 1-hour sessions (see Table 2) in a group-based format (between 3-8 participants). Two booklets are needed to deliver the SUPER-Ad program: the therapist manual and an adolescent workbook.

SUPER-Ad recommends the program be implemented by psychologists or school guidance staff (e.g., educational psychologists, assistants, etc.).

The SUPER-Ad program comes from cognitive-behavioral principles, in terms of incorporating psychoeducation module, making individuals aware of the link between thoughts, behavior and feelings; and testing for irrational anxiety-related beliefs and cognitive biases. The SUPER-Ad has five main cores: (1) To target common core risk factors for internalizing disorders, incorporating coping skills and strategies into adolescent repertoires. (2) To incorporate video feedback and cognitive preparation protocols as a program add-on. Program participants undergo some tasks which involve exposure to an audience or video camera, for instance, giving a speech in front of a video camera or doing role-plays to practice social skills. These tasks will be reviewed for each participant after completion. Some feedback will be provided by the audience (the rest of the group members). Additionally, each participant and the audience hold a discussion so as to discard irrational thoughts related to a bad performance in the task (Harvey, Clark, Ehlers, & Rapee, 2000; Rapee & Hayman, 1996; Rodebaugh, 2004). (3) To get adolescents ready to confront stressful situations: the program trains adolescents to face a wide variety of stressful situations deemed anxiety-provoking stimuli, teaching and modelling participants' behavior, following the cognitive-behavioral framework, and encouraging practice within real-life settings. (4) To promote behavioral activation: the program is eminently focused on providing practical skills that should be transferred to daily contexts. For this reason, the commitment to active practice is promoted for every skill taught to ratify the truthfulness and usefulness of thoughts and feelings. Moreover, participants must do homework exercises to spread behavioral activation into daily contexts. (5) To teach social skills: SUPER-Ad encompasses a specific module to teach and train social skills. Some skills are taught: starting conversations (with both known and unknown people), holding conversations, ending conversations politely, making requests, etc.

Table 2. The SUPER-Ad sessions and their main tasks.

Session	*Tasks*
Session 1. **Healthy lifestyle**	Introducing the program and members. Discussing healthy routines in terms of eating, sleeping and doing sport.
Session 2. **Self-esteem**	Working on self-esteem: from identifying perceptions about ourselves to discarding negative cognitive biases.
Session 3. **Thoughts and emotions**	Defining what an emotion is and providing valuable tools for recognizing feelings and thoughts (helpful vs. unhelpful ones).
Session 4. **Thoughts, emotions and behaviors**	Describing the connections between thoughts, feelings and behaviors. Introducing some tools for testing our thoughts (e.g., behavioral experiments).
Session 5 **Stress management skills**	Identifying the bodily and psychoemotional components of being stressed. Teaching strategies to manage stressful situations (e.g., controlled abdominal breathing).
Session 6 **Social skills training**	Teaching and practicing social skills.
Session 7 **Problem-solving skills training**	Incorporating problem-solving skills.
Session 8 **My team**	Clarifying when help is needed. Summarizing the program and reviewing the contents learned.

1.6. Summary: Gaps and Shortcomings in Adolescent Anxiety Research

A thorough review of studies in the field of adolescent anxiety has been conducted throughout the Background section and very valuable knowledge has been gained as a consequence of research in this field, although some knowledge gaps have yet to be filled in. A list of several of these gaps and other relevant shortcomings will be presented in this chapter. The following pages should show the correspondence between the listed items and the published studies included in this doctoral dissertation (see also Table 3).

Bearing in mind the traditional conceptualizations in the search for valid and accurate markers, linear models have always underlined the findings in most studies in the field of emotional disorders. Taking a DST framework may lead to a more comprehensive understanding of these distressing entities, providing important new conceptualizations in traditional research (e.g., the concept of multistability, fractal nature, etc.). Other disciplines and research fields have incorporated DST principles into their research topics with outstanding results (see West, 2006), and several early findings in the study of human emotion and psychopathology support the potential role of nonlinear measures as clinical tools for the diagnosis, assessment and therapeutic change monitoring (Llabres, Bornas, Noguera, Lopez, & Barcelo, 2005; Tiihinen et al., 1997; Yeragani et al., 2000). For this reason, Study 1 included in this thesis research aimed to identify nonlinear biomarkers for anxiety disorders (in adolescents as well as in other populations) but also for other emotional disorders (mood disorders).

Another important shortcoming to consider is the scarce amount of longitudinal designs to study anxiety in adolescence taking into account specific periods within the latter. As mentioned in previous chapters, developmental processes are ongoing across adolescence to become more mature bodily structures and physiological processes. As a consequence, emotional changes in adolescents may be seen at relatively short timescales (in months). Transversal studies may overlook the effects of these maturational processes and obtain misleading conclusions. In turn, longitudinal research studying entire adolescence through annual or biannual assessments may fail to capture the essential features of each specific period in adolescence. For this reason, a longitudinal approach focused on concrete periods should be taken when studying this turbulent period in life (see Cichetti, 1993; Hollenstein & Lougheed, 2013). Study 2 of this doctoral dissertation intended to examine the developmental course of anxiety symptoms from early to middle adolescence, and the potential influence of temperamental risk factors for anxiety disorder within it.

Furthermore, scientific knowledge in mental health stems from a particular and categorical standpoint which may hinder other distressing conditions being taken into account. Thus, a large gap exists for at-risk or subclinical conditions of anxiety because

leading diagnostic manuals drive research to full-blown clinical disorders. Mental health should tackle all sources of mental distress, despite some diagnostic criteria not being fulfilled. In this regard, a multi-level approach more focused on signs and pathological manifestations in the search for the related pathological processes may constitute a more suitable alternative. The RDoC initiative provides a framework that enables consideration of distressing conditions along continuums, rejecting a categorical approach to human healthcare and wellbeing (see Insel, 2014; Insel et al., 2010). The following three studies of this thesis dissertation were designed based on the RDoC guidelines. They thus provide some valuable findings from the negative valence system domain (anxiety symptomatology, explored at a self-report level) and the arousal system domain (cardiac function at a physiological level).

The study of physiological aspects in the manifestation of adolescent anxiety continues to present major shortcomings in terms of internal and external validity. For this reason, it would be invaluable to integrate evidence from controlled and ecological contexts. Firstly, and looking at internal validity concerns, laboratory studies often fail to find relevant heart-derived biomarkers for individuals along the anxiety continuum. Research with adolescents with ADs or at-risk profiles under resting conditions shows quite contradictory results (see Balle et al., 2013; Duncko et al., 2006). Therefore, useful laboratory paradigms (emotion induction protocols) are needed in the search for these biomarkers. Moreover, other potential biomarkers (nonlinear measures) should be explored. Secondly, a shift toward a more ecological way of research should be undertaken in an attempt to bring research and real life closer together, and gain more external validity. Study 3 of this doctoral dissertation aimed at investigating the discriminating power of some heart-derived measures (linear and nonlinear) to distinguish between adolescents with high and low anxiety symptomatology under ecological conditions. In turn, Study 4 intended to examine how different cardiac reactivity in adolescents was with increasing trajectories of anxiety symptomatology under emotion induction conditions (a stressor confrontation task) in comparison to adolescents without such trajectories.

Finally, a large gap has been identified in terms of adolescent anxiety prevention. As mentioned in a previous chapter, preventive programs for anxiety in adolescence showed (in the politest terms) modest effects in the reduction of symptomatology and low methodological controls (see Garcia-Escalera et al., 2016; Werner-Seidler et al., 2017). Tackling maladaptive anxiety in adolescence must be made a key concern for mental healthcare providers and institutions due to its dramatic impact on current and subsequent periods in life. The delivery of the school-based SUPER-Ad program may be effective in reducing anxiety symptomatology (or in preventing the escalation of anxiety) as it preserves some important elements that are highly related to good prevention outcomes: being a school-based intervention, having a transdiagnostic nature, and having a reference program (SSL) that has shown good results in samples of children (see Essau et al., 2014). In this regard, Study 5 aimed to test the effectiveness of the SUPER-Ad program for ameliorating anxiety symptomatology across adolescence.

In short, this thesis dissertation essentially aims, on the one hand, to contribute to fill these research gaps by means of finding relevant markers that enable adolescents along the continuum of anxiety to be identified, and on the other, to provide and test a prevention tool for ameliorating adolescent anxiety symptomatology.

Table 3. Shortcomings in the field of adolescent anxiety research and potential solutions.

Shortcoming	Potential way of solution	Doctoral dissertation study
The lack of accurate biomarkers for anxiety and other emotional disorders	To explore nonlinear measures as another way to find biomarkers	**Study 1.** Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study
The great proportion of transversal, static studies or longitudinal studies vaguely focused on the whole adolescence	To conduct longitudinal studies (with multiple points of assessment) focused on concrete periods of adolescence	**Study 2.** Anxiety from early to middle adolescence: Developmental trajectories and associations with temperamental factors
The low generalizability of results found in laboratory settings to everyday life contexts	To conduct controlled studies within ecological conditions	**Study 3.** Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment
The lack of accurate heart-derived biomarkers for identifying individuals along the anxiety continuum	To implement laboratory protocols (emotion induction protocols) to detect differential characteristics among adolescents with anxiety	**Study 4.** Impaired cardiac profile in adolescents with an increasing trajectory of anxiety when confronting an acute stressor
Prevention programs for adolescent anxiety show modest effects	To deliver programs with empirically supported components: the SUPER-Ad	**Study 5.** Trajectories of anxiety symptoms in adolescents: Testing the model of emotional inertia

Publications 2

2.1. List of publications

Study 1. De la Torre-Luque, A., Bornas, X., Balle, M., & Fiol-Veny, A. (2016). Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. *Neuroscience & Biobehavioral Reviews, 68*, 410-422. doi: 10.1016/j.neubiorev.2016.05.023 (JCR® Impact Factor = 8.30)

Study 2. De la Torre-Luque, A., Fiol-Veny, A., Nelemans S. A., Balle, M., & Bornas, X. Anxiety from early to middle adolescence: Developmental trajectories and associations with temperamental factors. Currently under review in *Journal of Abnormal Child Psychology* (JCR® Impact Factor = 3.61)

Study 3. De la Torre-Luque, A., Fiol-Veny, A., Balle, M., & Bornas, X. (2016). Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment. *International Journal of Clinical and Health Psychology, 16*, 287-294. doi: 10.1016/j.ijchp.2016.04.002 (JCR® Impact Factor = 2.57)

Study 4. De la Torre-Luque, A., Fiol-Veny, A., Bornas, X., Balle, M., & Llabres, J. (2017). Impaired cardiac profile in adolescents with an increasing trajectory of anxiety when confronting an acute stressor. *European Child and Adolescent Psychiatry*. doi: 10.1007/s00787-017-1009-8 (JCR® Impact Factor = 3.29)

Study 5. Bornas, X., De la Torre-Luque, A., Fiol-Veny, A., & Balle, M. (2017). Trajectories of anxiety symptoms in adolescents: Testing the model of emotional inertia. *International Journal of Clinical and Health Psychology, 17*, 192-196. doi: 10.1016/j.ijchp.2017.01.002 (JCR® Impact Factor = 2.57)

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Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study

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ABSTRACT

This meta-analysis aimed at gathering and summarising the findings on nonlinear biomarkers in the field of emotional disorders under the hypothesis that diseased systems show lowered complexity and hence less flexibility to adjust daily contexts. Scientific manuscripts from 1970 to 2014 were reviewed, 58 articles were analysed, and independent meta-analyses on anxiety disorders, bipolar disorders, and depressive disorders were conducted. Results revealed that anxious patients exhibited lower complexity than controls ($p < 0.05$) despite panic patients showed more irregular respiratory activity. Inconclusive results were found for bipolar patients but pointed to higher randomness when suffering manic episodes. Finally, depressed patients showed a loss of complexity in the cardiac system and a loss of orderliness (despite a higher complexity) in brain and stress-related hormonal systems. As a conclusion, our findings highlight that either a loss of complexity or a loss of ordered complexity characterise the physiological systems of patients with emotional disorders. Several considerations for complexity, its related measurements, and suggestions for further research are discussed.

Contents

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1. Nonlinearity, complexity, and disease

Along with the traditional search for biomarkers for psychological diseases, such as frontal EEG alpha band asymmetric activity in depression or diminished heart rate variability in anxiety disorders, in the last decades the search has been growing for biomarkers arising from the Nonlinear Dynamical Systems (NDS) theory approach to psychopathology, which best captures the real, non-static conditions where physiological and behavioural systems evolve over time. Strictly speaking, "nonlinear" refers to the lack of proportionality between a stimulus and the system's response to that stimulus. However, the word "nonlinear" is currently used in a broader sense to refer to the large array of models and methods (chaos, fractals, synergetics, etc) under the NDS term umbrella (Guastello et al., 2009). This broader meaning is the one we adopt in this paper, and it is close to the meaning of the word "complexity". In a review of complexity assessment in physiological systems, Burggren and Monticino (2005) included chaotic systems' characteristics (e.g. sensitive dependence on initial conditions), informational theory advances (e.g. complex systems usually have higher entropy rates than simpler ones), and emergent or self-organised behaviour under the "complexity" label. Thus, the terms "nonlinear" and "complex" are often used interchangeably to refer to systems exhibiting one or more of these properties.

1.1. Assumptions guiding the search for nonlinear biomarkers

The search for nonlinear biomarkers has been based on two main assumptions. First, it has already been assumed that physiological systems are complex (Goldberger, 1996; Goldberger et al., 2002), and, therefore, these systems cannot be fully understood by breaking them down into simpler components. One milestone in the process of adopting that perspective of complexity was the introduction of fractal physiology by West (1990), after Benoit Mandelbrot's discovery of fractals (Mandelbrot, 1967). Numerous studies demonstrating the nonlinear and complex nature of the cardiac system, the respiratory system, or the human gait were compiled by West (2006). Regarding the central nervous system, the work of Freeman (1999) was crucial in stressing the complex nature of brain dynamics on several time scales, and a state of self-organised criticality (Bak et al., 1987) seems to be the brain's default mode of functioning (Chialvo, 2010; Garrett et al., 2013; Pittman-Poletta et al., 2013; Werner, 2010).

The second assumption is that diseased systems (and also aged systems) usually show reduced complexity (Guastello, 2004; Pikkujamsa et al., 1999; Stiedl et al., 2009). In addition to a large body of favourable empirical research (Costa et al., 2002; Guastello et al., 2009; Peng et al., 1995), there are theoretical reasons to think in this way. Human physiological systems continuously interact with a changing environment, and successful adaptation to these conditions requires flexibility, i.e. the ability to cope with the huge amount of demands coming from that environment. This interaction needs much more sophisticated mechanisms than a memory-less thermostat (homeostasis). Specifically, these systems have to operate on multiple time scales, since merely responding in a single real-time scale is not sufficient. Even at rest these systems do not remain stable, that is to say, they do not fluctuate on a single time scale (contrary to the predictions of homeostasis) but show scale invariance: statistically similar patterns of fluctuations exist on multiple time scales. Because of this, scaling techniques (e.g. detrended fluctuation analysis) are widely used to assess the complexity of physiological systems. Temporal multi-stability is, therefore, a main characteristic of complex physiological systems, and any loss of complexity diminishes the system's ability to cope flexibly with the environment.

1.2. Diminished complexity as sign of disease

It would be reasonable to expect, therefore, that psychological syndromes, such as depression or anxiety, be linked to diminished complexity of related physiological systems. Just as Costa et al. (2002) assumed the proposal that loss of complexity could be a generic feature of pathologic dynamics in medical diseases, this loss of complexity could be a biomarker for psychological diseases. The number of studies looking for complexity losses in patients suffering from a wide range of disorders (anxiety, bipolar disorders, post-traumatic stress disorder, depression, schizophrenia, etc) has been growing in the last two decades, albeit slowly. However, findings derived from this literature still remain rather disperse due to conceptual and methodological problems.

Perhaps the most important problem has to do with the fact that there is no single, direct measure of the complexity of a physiological system (for a review, see Burggren and Monticino, 2005). Complexity is inferred from one or more measures taken within one or more "domains of variability" (Bravi et al., 2011), that is to say, the informational domain or the scale invariant domain. Examples of metrics in the informational domain are approximate entropy (Pincus, 1995), sample entropy (Richman and Moorman, 2000) or multiscale entropy (Costa et al., 2002), all of them measuring the rate of new information generated by an evolving system. As complex systems are characterised by their highly unpredictable long-term behaviour, a system's entropy (in the informational domain) can be measured and used afterwards to infer the degree of complexity (in fact, unpredictability) of the system. However, non-complex systems may display random (and, therefore, unpredictable) behaviour, so that high entropy could be misleading – both random and complex systems may show unpredictable behaviour. Regarding specific measures in the scale invariant domain, similar problems may arise. Examples of metrics in this domain are the correlation dimension (estimation of the system's degrees of freedom), the largest Lyapunov exponent (a measure of the system's sensitivity to initial conditions) or the scaling exponents from detrended fluctuation analysis (measuring temporal multistability). Considering that last metrics for instance, the short-term scaling exponent used to assess the complexity of the heart beat should be close to 1, thus approaching the so-called 1/f scaling phenomena. The precise meaning of exponents slightly lower (or higher) than 1 remains far from clear (though large deviations from unity have straightforward implications in the medical area; Peng et al., 1995). We suggest that many results reported in scientific papers should be interpreted cautiously as indicators of the degree of complexity of the relevant system.

1.3. Aims of this meta-analysis: testing the complexity-loss hypothesis in emotional disorders

Based on our assumptions, this meta-analytic review aimed to elucidate whether physiological systems of people suffering from

anxiety-related disorders and mood-related pathologies (depressive and bipolar disorders) actually show reduced complexity (for non-physiological NDS-oriented studies on the same disorders, see for instance Katerndahl and Wang, 2007). These disorders were chosen because of their substantial influence on successful contextual adjustment and their high prevalence rates (American Psychiatric Association, 2013; De Graaf et al., 2012). A wide variety of NDS-based measures has been used to evaluate the complexity of the cardiac system, the brain system and the endocrine system (amongst others) of patients suffering from these disorders. Following meta-analytic methodology enables us to summarise and extract conclusions across the studies based on quantitative empirical data, even if findings arising from them may show a certain degree of inconsistency (Evans, 2003; Rice, 2008). Hence, this meta-analytic review should be seen as an attempt to synthesise the results obtained by studies using those measures. For instance, the study attempts to answer questions such as, "Is there any NDS-based measure to characterise the cardiac system of anxious patients?" From the meta-analytic review of the studies, focusing on very specific questions (such as, "Are patterns of cortisol secretion in anxious individuals more unpredictable than patterns showed by non-anxious people?"), we should be able to answer more general questions such as the first one. In addition to the main goal of the study, we investigated the moderating influence of several sample-related factors (e.g. age, gender, etc) as well as setting-related factors (physiological systems measured and assessment context, amongst others) on the results of the studies.

2. Methods

2.1. Selection criteria for the studies

Criteria used to select studies were proposed in keeping with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) suggestions (Moher et al., 2015). Therefore, studies had: a) to be empirical comparative studies (case-control studies or cohort studies) that included either people with a diagnosis of mood disorder (depressive or bipolar-like) or another anxiety disorder, according to standardised diagnostic manuals (any version of DSM, ICD, or others); b) to include healthy (control) groups; c) to analyse data from more than 5 participants each group; d) to analyse any human physiological system through nonlinear measures between groups; and e) to be published as an original article in scientific press from 1970 to 2014 and written in either the English, Spanish or Catalan languages. Papers that showed results from either subclinical samples, patients in remission from these disorders, or non-pure samples (participants with anxiety and/or mood disorders within a wider sample with other disorders) were ruled out. Manuscripts which did not provide enough data to extract the required single effect size were also removed.

2.2. Search and selection procedure

Papers were located by following a two-way approach. Firstly, the main scientific databases were consulted (ascendant search approach): Cochrane Plus Database, databases provided by EBSCOhost server (FRANCIS, PsycInfo, PsycARTICLES, CINAHL, and E-Journals); databases provided by OvidSP server (Ovid Nursing Database, all OVID journals, and NASW Clinical Register); PubMed, PubPsych, Scopus, and Web of Science. The database search was conducted between October and November 2014. Queries were created based on two sets of elements (see Table 1): the first set referred to keywords that identify emotional disorders which were established by reviewing standardised manuals for mental disorders (different versions of DSM and ICD). The second set of keywords included concepts derived from the NDS theory and collected by following expert criteria in keeping with the classification by Bravi et al. (2011).

Table 1
Keywords used for this meta-analysis.

Clinical Keywords	NDS-based keywords
Affective disorders	Nonlinear, non-linear, nonlinearity
Seasonal affective disorder	Dynamic (dynamical) Systems
Emotional disorders	Attractor
Mood disorders	Phase space
Humor disorders	State space grids
Depression	Entropy (approximate entropy,
Depressive episode	sample entropy, Shannon
Bipolar disorder	entropy. . .)
Mania (maniac episode)	Fractal (dimension) (estimation)
Dysthymia (dysthymic disorder)	Scaling, scaling exponent, scale
Cyclothymia (cyclothymic	invariance
disorder)	Power-law correlation
Anxiety	Allometric
Anxiety disorders	Long range temporal correlations
Situational anxiety	Detrended Fluctuation Analysis
Worry	Symbolic Dynamics
Stress	Orbital decomposition
Stressor	Synchronization
Traumatic events	Self-organization
Burnout	Criticality
Fear	Self-organized criticality
Phobia	
Neurosis (or neurotic)	

Note. All terms were crossed each other to make specific search queries with all the combinations.

Secondly, the descendant search approach was followed. This consisted of revising the reference list of each selected manuscript in order to locate other manuscripts which were not indexed in scientific databases.

In order to select the studies, two reviewers had to state independently that each paper satisfied the selection criteria. Therefore, articles were screened through an initial review of title, abstract, and keywords (screening stage). Afterwards, they proceeded to read the manuscript in full to ratify the selection (full-reading stage).

2.3. Coding the studies

Each selected manuscript was read in full and relevant data (moderator and dependent variables) were extracted from each one by a reviewer. Papers were coded using a database developed for these purposes. Afterwards, another reviewer tested coding tools by reading and categorising a random selection of the study sample. This selection encompassed 25% of the total sample of articles. As a result, 92.90% of the relevant data was equally coded by the two reviewers, χ^2 (1) = 133.71, $p < 0.001$. Disagreements were resolved by discussion.

2.3.1. Coding moderator variables

Four types of moderators were assessed: a) sociodemographic (age, gender and body mass index); b) the methodological quality of the studies, measured by means of the Newcastle – Ottawa Quality Assessment Scale (NOQAS; Wells et al., 2014) which provides a quantitative score from 0 ('absence of methodological controls') to 9 ('maximum level of methodological controls'); c) clinically-related moderators, i.e. comorbidity and sample medication. Comorbidity was defined as the presence of a comorbid condition in conjunction with the relevant clinical symptomatology and clearly mentioned in the primary study. Sample medication referred to whether the clinical subjects were medicated to control the related symptomatology when the study was ongoing.

Table 2
Moderating factors and categories for this meta-analysis.

Variable	Categories	Definitions
Sociodemographic[a]		
Age		Mean age of participants
BMI[b]		Mean body mass index of participants
Gender		Proportion of male participants within the sample
Methodological quality[a]		How strong controls were applied within the research protocol in keeping with NOQAS
Clinical-related aspects		
Comorbidity	No comorbidity	No disease/disorder described as concomitant
	Physical	Organic diseases comorbid
	Psychological	Psychiatric or neurologic disorders comorbid
	Both	
Sample Medicalization	Free-of-medication	Less than 50% of patients medicated
	Medicated	More than 50% of patients medicated
Environment of measurement		
Activity measured	Cardiac	Heart-derived functioning taken from recording its derived electric potencial
	EEG dynamics	Cerebral activity taken from registering electrical potential over the scalp of the head
	Neuroimaging	Brain activity taken through brain imaging devices
	Muscular	Muscular activity registered by using electromyographic devices
	Electrodermal	Activity showed by changes on the electric properties of the skin
	Electrooculographic	Activity derived from the muscles around the eyes
	Respiratory	Activity proper of respiratory System
	Hormonal	Activity derived from the endochrine System
	Self-reported	Integrated activity reported as responses to interviews, questionnaires, and so on
	Others	
Assessment context	Laboratory	Measurement within laboratory/hospital settings
	Ecologic	Measurement taken under daily life contexts
Task measured	Resting	Participants were requested to sit quiet and relaxed, breathe regularly and move as little as possible. For EEG studies, it distinghised between resting with closed or opened eyes
	Experimental tasks	Participants should perform some tasks or be exposed to experimental tests
	Others	Mixed protocols, or other types of tasks
Duration of assessment	Short registration	Less than 1 h of recorded activity
	Long registration	Activity taken for more than one hour

Note. NOQAS = Newcastle–Ottawa Quality Assessment Scale (Wells et al., 2014).
[a] Variables that cover these types of moderators were measured continuously and they do not therefore encompass any category.
[b] This variable was considered just for studies measuring cardiac and respiratory activity.

d) Finally, four categorical variables relating to the measurement environment were taken into account: the physiological system on which the nonlinear measures had been calculated; the assessment context and the task that participants had to perform during physiological monitoring, and the moderating role of the duration of monitoring. Features and categories considered for all of the aforementioned variables are showed in Table 2.

2.4. Computation of effect sizes

As dependent variables, the effect sizes extracted were taken by comparing the scores of the clinical group vs. the control, for each NDS-based measure. These measures were grouped according to the five domains proposed by Bravi et al. (2011), to avoid effects of dependency among the observations: a) nonlinear statistical measures which enable study of statistical characteristics of nonlinear processes of a biological system (i.e. symbolic dynamic features, form factors, etc.); b) nonlinear geometric measures which are based on extracting shape-related information on system behaviour in a time series (i.e. Poincaré plots features, recurrence plot features, etc.); c) nonlinear energetic measures which are focused on the power of the dataset within a time series (i.e. multiscale time irreversibility); d) nonlinear informational measures which allow quantification of the degree of irregularity/new information inherent to the order of the elements in a time series (i.e. entropy-like measures, similarity indices, etc.); and e) nonlinear invariant measures which enable the study of system properties that remain constant either over time or space (i.e. fractality, Lyapunov exponents, etc.).

2.5. Data analysis

Separate meta-analyses were conducted for each type of emotional disorder considering random effects for all of them.

First of all, it was ensured that all single effect sizes within the NDS categories provided the same information. As pointed out earlier, the relationship between specific measures (such as scaling exponents) and complexity levels is not direct. A careful analysis was therefore required in many cases to interpret the results appropriately. Changing the sign of the effect size was a simple way to align interpretations (i.e. complexity increases or decreases) and to avoid controversies among measures within the same domain. Therefore, for instance, information index (S_0) scores were sign-reversed in order to show results in the same direction (higher values meaning more information).

A single effect size was then calculated for each study. These calculations were based on the standardised difference between group means. Hedges' *g* statistic was calculated for each study as this statistic shows lower levels of bias, even if the sample size is small. The unbiased estimator of *g* based on the gamma function was therefore applied (see Botella and Gambara, 2002; Hedges and Olkin, 1985).

Statistical assumptions required to carry out meta-analytic calculations were tested afterwards. A multivariate normality test was performed and independence among single effect sizes was ensured by following specific rules: a) only one nonlinear measure effect size was incorporated for each category of dependent variables. In this way, the effect size for the most frequently used measure among the studies would be selected. If all measures

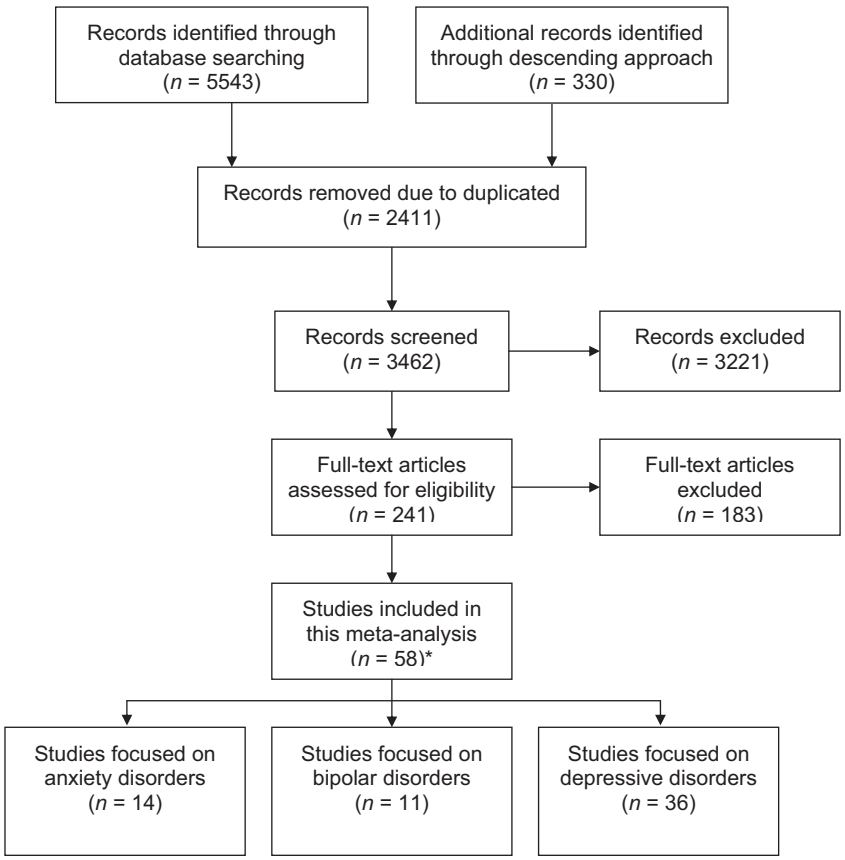


Fig. 1. Flow diagram of study selection.
Note. *The lack of congruence between this box and the next ones is because there were two selected studies which were focused on several psychological disorders: Baumert et al. (2009) studied groups with depression and anxiety groups and Moon et al. (2013) incorporated sample with anxiety, depression and bipolar-disordered groups.

reported within a study were equally frequent, one was randomly selected. b) Only one clinical group was incorporated on a random basis. c) Only one activity and task measured were incorporated, being selected randomly.

Overall effect sizes were weighted by their inverse of variance, which is obtained via single intra-study variability and an estimate of the between-study variance (Botella and Gambara, 2002). Moreover, a 95% confidence interval and the contrast test based on the Z_c statistic were calculated in order to ensure that each overall effect size was significantly different from zero. The overall effect size was interpreted following Cohen's (1988) recommendations and taking into account that overall effect size referred to higher values for this measure in favour of the clinical group. Conversely, negative overall effect size pointed to higher levels for control group participants. Homogeneity between studies was then analysed by means of the Q_t statistic (Hedges and Olkin, 1985) and the I^2 index (Higgins and Thompson, 2002) in order to delimit if differences among studies were due to systematic sources of variation (due to the influence of moderating factors). To be precise, analyses based on analogues of analysis of variance (ANOVA) were conducted to explore the influence of categorical moderator variables on these variations (see Hedges and Pigott, 2004). Forest plots were also attached to complement this analysis visually. Meanwhile, quantitative model fitting was also used to determine the explanatory loading of the quantitative moderators on criterion by using weighted multiple meta-regressions. Model estimations were based on maximum likelihood methods (Botella and Gambara, 2002; Hedges and Pigott, 2004).

Finally, Egger's regression test was used to assess the presence of publication bias (Egger et al., 1997; Ferguson and Brannick, 2012).

SPSS v. 19 (script by Lipsey and Wilson, 2001) and R x64 3.0.1 (METAFOR Package) were used to conduct all the statistical and graphical analyses.

3. Results

Fig. 1 depicts the steps followed to select the sample of articles. A total of 5873 original papers were reviewed. Once the screening and in-depth reading stages were conducted, a sample of 58 articles was considered. Study reviewers agreed on sample selection.

All of the selected papers were written in the English language. Most were published after 2000 (89.65% of the articles). There was one study (Moon et al., 2013) which had the three targeted clinical groups within its sample; and another (Baumert et al., 2009) which studied depression and anxiety groups. Regarding the rest of papers, 12 studies worked with anxiety-disorder patients, 10 analysed NDS-based measures in subjects with bipolar disorder, and 34 studies considered depressive syndromes. The features of the studies included can be explored in the Appendix A of Supplementary information.

3.1. Anxiety disorder studies

The mean number of participants for the studies focusing on anxiety-disorder patients were 45.36 individuals ($sd = 40.19$). The average age of participants was 31.73 years old ($sd = 8.84$). These studies showed a methodological quality score of 5.57 points, on average ($sd = 1.65$). Medication-free subjects ($k = 8$) and no explicit comorbidities ($k = 14$) were mainly reported. The activity measured

Table 3
Summary of overall effect sizes for the outcomes and disorders considered.

	k	g (CI_{95})	Q_t	I^2
Anxiety-disorder studies				
Informational measures	4	0.69** (0.34, 1.03)	3.75	19.93
Invariant measures	12	0.80* (0.18, 1.43)	82.28**	86.63
Bipolar-disorder studies				
Informational measures	6	−0.02 (−0.60, 0.57)	26.35**	81.02
Invariant measures	6	0.08 (−0.41, 0.56)	20.19**	75.23
Depressive-disorder studies				
Statistical measures	5	0.34 (−0.14, 0.83)	12.50*	68.00
Informational measures	24	−0.02 (−0.43, 0.39)	210.76**	89.56
Invariant measures	16	−0.05 (−0.42, 0.35)	94.18**	84.07

Note. This table collects dependent variables categories in which it was possible to calculate overall effect sizes.
k = number of studies; g = weighted overall effect size; CI_{95} = 95% confidence interval for the overall effect size; Q_t = heterogeneity Q statistic; I^2 = Higgins and Thompson's heterogeneity statistic (in percentage).
* $p < 0.05$.
** $p < 0.01$.

in these studies mainly stemmed from cardiac functioning ($k = 7$), within laboratory settings ($k = 11$), performing different tasks ($k = 6$ for the category 'others'), and using short-term monitoring ($k = 8$).

Regarding dependent variables, it was found that two studies analysed statistical measures (measures belonging to the statistical variability domain), four studies used informational measures and 12 studies included invariant measures. Single effect sizes for all studies are depicted in Fig. 2.

Taking into account the NDS-based measures in the statistical domain, there were not enough studies to extract an overall effect size based on random effects for anxiety disorders. Single effect sizes were around $g \approx 0.00$ found by Baumert et al. (2009) and $g = 0.69$ by Yeragani et al. (2000). Both studies recruited patients with panic disorder and analysed cardiac activity. Regarding measures in the informational domain, single effect sizes ranged between $g = [0.15, 0.99]$, with intra-study variability around $w_i = [3.73, 12.14]$. For these measures, an overall medium effect size was found that was significantly different from zero, $Z_c = 3.92$; $p < 0.01$. In the invariant domain-related measures, single effect sizes were between $g = [-0.72, 4.72]$ and their intra-study variability $w_i = [1.26, 14.01]$. In this case, a significantly large overall effect size was also found, with $Z_c = 2.52$; $p > 0.02$. Details relating to the overall effect sizes for each NDS-based measure categories are displayed in Table 3.

Homogeneity between studies was then tested for each category of dependent variables, finding wide heterogeneity in studies that included invariant measures. This was accounted for by conducting model fitting analyses. Hence, analyses based on analogues of ANOVA and multiple meta-regressions were carried out, but no significant models could be derived from these analyses.

Finally, the influence of the publication bias on these results was tested. As a result, Egger's regression test revealed a significant influence of this bias on invariant-measure overall effect size ($Z = -2.36$, $p < 0.05$). This result means that significant levels of asymmetry were found for results obtained in this domain of variability.

3.2. Bipolar disorder studies

Studies focusing on bipolar disorders were carried out on an average of 57.55 participants (sd = 40.72) and all of them were adults (average age was 36.05 years old, $sd = 7.18$). The average methodological quality of those studies was 6.18 points ($sd = 1.54$). All of them were conducted with medicated subjects and comorbidities were not reported. The most common activity measured was cardiac ($k = 3$), within laboratory settings ($k = 9$), under resting conditions ($k = 5$) and using short-term recordings ($k = 7$).

Regarding the NDS-based measures, one study analysed symbolic dynamics, two included geometric measures, six studies analysed informational measures and another six included invariant measures (see Table 3). For geometric measures, single effect sizes were distributed between $g = [-0.83, 0.08]$ and their intra-study variability was around $w_i = [7.08, 13.70]$. With respect to informational measures, single effect sizes ranged from $g = [-1.36, 0.85]$ and intra-study variability, $w_i = [3.99, 16.06]$. A non-significant overall effect size was shown regarding these types of measures, $Z_c = -0.06$; $p > 0.95$ (see Table 3). On the other hand, single effect sizes for the invariant measures ranged between −0.60 and 1.23, with intra-study variability between 4.99 and 16.88 and overall effect size was again non-significant, with $Z_c = 0.31$; $p > 0.75$.

Results arising from homogeneity tests revealed wide heterogeneity among studies in terms of the overall effect sizes for informational, as well as invariant, measures. Nonetheless, model fitting could not be tested due to the low sample size and the great variability among studies. Due to the importance of this type of sample in bipolar disorders, we tried to visualise whether a significant overall effect size arose from samples in acute episodes. As a result, this was just possible for informational measures ($k = 3$), with a non-significant effect size being observed due to the high variability among studies, $g = 0.22$ ($se = 0.54$), $p < 0.38$; Q_t (2) = 16.57, $p < 0.01$.

Finally, Egger's regression test indicated an absence of publication bias influence ($p > 0.05$).

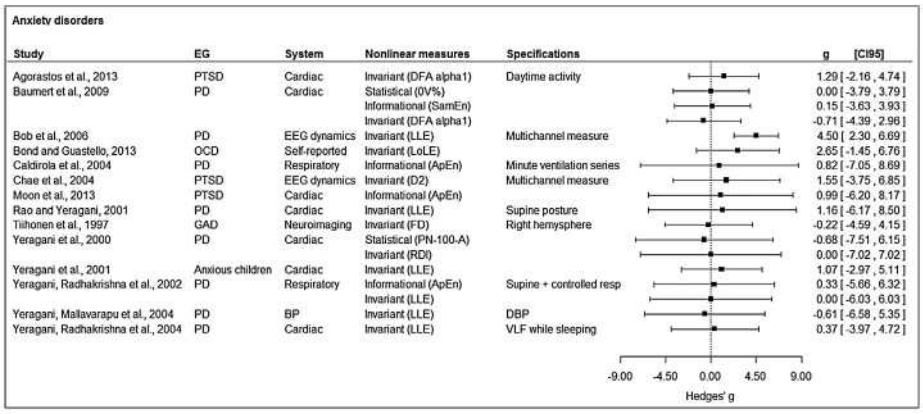
3.3. Depressive disorder studies

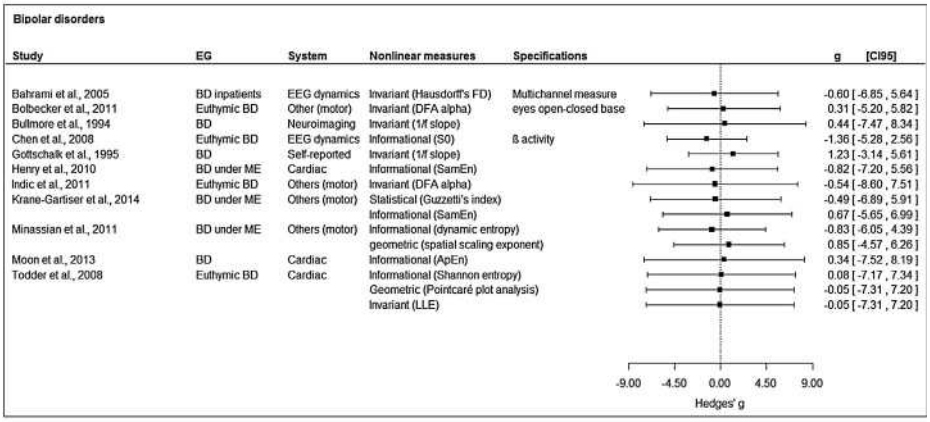
These 36 studies included samples which were made up of an average of 50.03 participants ($sd = 39.31$) with a mean age of 38.30 ($sd = 9.84$). On average, they showed a methodological quality of 6.53 ($sd = 1.36$). Additionally, it was shown that the greater number of these studies consisted of medication-free subjects ($k = 26$), monitoring most commonly cardiac activity ($k = 15$) within laboratory settings ($k = 29$), under resting conditions ($k = 16$), and based on short-term recordings ($k = 26$). With respect to NDS-based measures, five studies included statistical measures as dependent variables; two studies included geometric ones; 23 analysed informational measures; only one included energy measures and 16 included invariant measures (see Appendix A of Supplementary information).

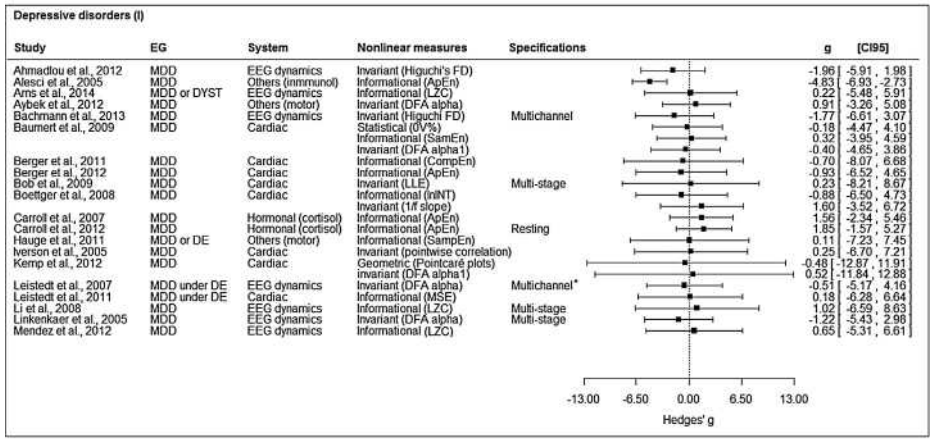
Overall effect sizes were calculated for statistical, informational, and invariant measures (see Table 3). In relation to the measures within the statistical domain, single effect sizes ranged from $g = [-0.23, 0.90]$, with intra-study variability between $w_i = [4.06, 27.31]$. Regarding informational measures, study effect sizes ranged from −4.83 to 3.06 and their related intra-study variability between 1.15 and 29.57. Considering invariant measures, single effect sizes were between −1.96 and 1.60 with intra-study variability ranged from 3.48 to 39.78. Overall effect sizes for these categories of nonlinear measures were not significantly different from zero ($p > 0.05$ for all cases). Effect sizes for geometric measures fluctuated from −0.68 and −0.48; and −0.26 for the study that included energy measures (Tonhajzerova et al., 2012).

Homogeneity tests showed a lack of homogeneity among studies for these three categories of measures (see Table 3). Therefore, great variability in study effect sizes testing for explanatory models was revealed.

Categorical models were initially tested for nonlinear statistical measures. In this case, it was not possible to create different categories among studies which included these measures. However, it was noticed that there were three studies which considered cardiac activity and we decided to extract the overall effect size







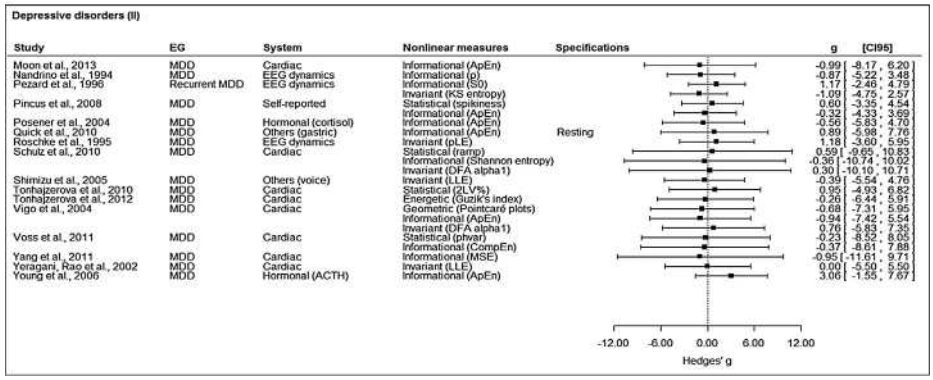


Fig. 2. Effect size distribution among studies.
Note. Hedges' statistic was used to account for effect size (error bars depict its 95% confidence interval).
'Specifications' refers to the definite time series used to calculate single effect sizes for that study within this meta-analysis.
BD = bipolar disorder; DE = depressive episode; DYST = dysthymia; GAD = generalized anxiety disorder; MDD = major depression; ME = manic episode; OCD = obsessive-compulsive disorder; PD = panic disorder; PTSD = posttraumatic stress disorder. DBP = diastolic blood pressure; BP = blood preassure; immunol = Immunologic;

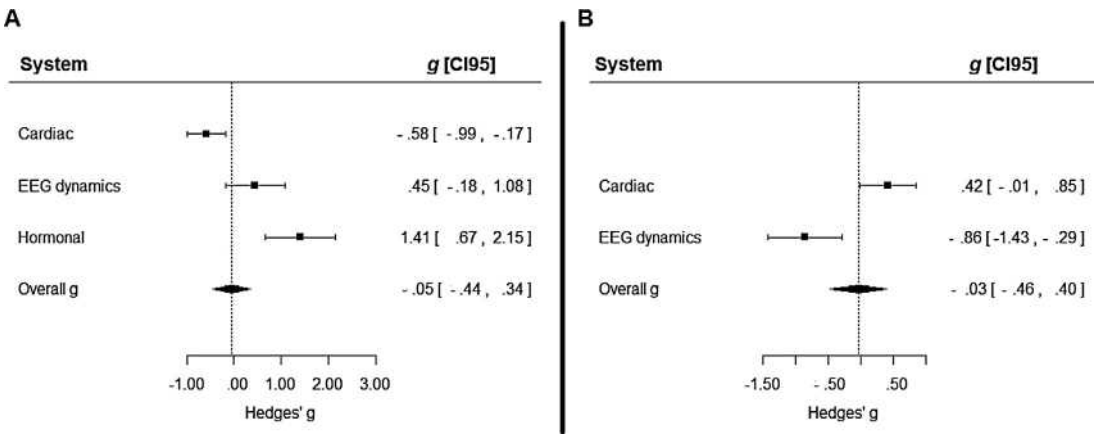


Fig. 3. Forest plots for the depressive-disorder studies.
Note. This figure shows studies focused on depressive samples which analysed nonlinear informational measures (graph A) and nonlinear invariant measures (graph B), in function to activity measured.
g = weighted overall effect sizes; CI95 = 95% confidence interval for Hedges' *g*.
More positive and higher Hedges' *g* state higher complexity for depressive groups in comparison to controls when considering informational measures. Conversely, more positive and higher Hedges' *g* indicate lower complexity (higher invariance) for depressive groups in comparison to controls when considering invariant measures.

for this set of studies. As a result, $g = 0.10$ ($IC_{95} = -0.52, 0.73$) with $Z_c = 0.33$, $p > 0.05$ were observed. This set of results kept showing high heterogeneity in studies as shown by $Q_t\ (2) = 8.21$, $p < 0.02$.

Afterwards, categorical models were conducted for informational measures. As a result, a significant model was found, with $Q_B\ (2) = 22.54$; $p < 0.01$. This included the activity measured as a categorical moderator but considering just three categories, due to the low sample size of the others: cardiac activity ($k = 10$), EEG dynamics ($k = 5$) and hormonal activity ($k = 4$). Overall effect sizes for the categories are displayed in Fig. 3. Studies that analysed cardiac activity showed a negative medium effect size. When EEG dynamics were considered the overall effect size was smaller but positive. Positive, large overall effect size was found when hormonal activity was analysed.

Categorical models were tested for invariant measures afterwards. In this case, results were fairly similar to those observed for informational measures, revealing a significant model with the same categorical moderator (activity measured), with $Q_b\ (2) = 11.82$, $p < 0.01$. In this case, just two categories were considered due to the low sample size for the others: cardiac-activity studies ($k = 8$), with a positive overall effect size; and EEG-dynamic studies ($k = 6$), with a negative overall effect size (see Fig. 3).

No significant meta-regressive models were found to explain the overall effect sizes of either statistical, informational or invariant measures.

Finally, publication bias was tested. As a result, Egger's regression test revealed a significant influence of that bias on the results which arose when the informational measures were analysed, $Z = 2.21$; $p < 0.03$.

4. Discussion

Emotional disorders increasingly lead to significant distress and life interference for people and specific biomarkers may guide research and intervention pathways for such diseases. Some pieces of evidence have been obtained from the NDS framework with disperse results. This meta-analysis aimed at gathering and drawing overall conclusions from these findings. In this regard, it was proposed to shed light on the discriminatory role of nonlinear measures to distinguish between emotionally-disordered and healthy, non-disordered systems, in an attempt to search for biomarkers with robust relevancy for research and clinical practice. Moreover, it was aimed at determining how some moderators influence this discriminatory capacity.

First of all, we should say that the bulk of results in this meta-analysis support the general hypothesis that diseased systems show reduced complexity, but some of these must be interpreted cautiously as the word "complexity" does not have a single meaning. Therefore, we will discuss the results for each one of the three types of disorders included in the meta-analysis (see Table 4 for an overview of the conclusions arising from our results) and then we will go back to the "loss of complexity" topic to close discussion of the results.

Results from studies focusing on anxiety disorders were in line with our hypotheses when taking statistical and invariant measures into account. Anxiety-induced, altered and biased information processing has been endorsed by the lower brain complexity of anxious participants, such as under resting conditions and when retrieving stressful memories (Bob et al., 2006; Chae et al., 2004). This generalised loss of brain complexity has been interpreted as neuronal inactivation or loss of dynamic brain responsiveness, and may be due to anatomical changes observed in frontal, temporal, cingulate and subcortical areas of anxiety-disordered patients (for a review, see Martin et al., 2009). On the other hand, sympathetic hyperactivity and vagal withdrawal, the main autonomic features involved in anxiety disorders (Friedman, 2007; Thayer and Lane, 2009), have been strongly connected to reduced complexity when monitoring the cardiovascular function in anxious patients (Baumert et al.,

resp = respiration; VLF = very low frequency power.
Nonlinear measures: 0V% = patterns with no variation; 2LV% = patterns with two like variations; ApEn = approximate entropy; CompEn = compression entropy; D2 = correlation dimension D2; DFA = detrended fluctuation analysis exponents; FD = fractal dimension; KS entropy = Kolmogorov-Smirnov entropy; LLE = largest Lyapunov exponent; lnINT = inherent information curve index (in loglineal scale); LoLE = local Lyapunov exponent; LZC = Lempel-Ziv complexity index; MSE = multiscale entropy; phvar = difference between two successive RR-intervals lower than a specified limit; pLE = principal Lyapunov exponent; PN-100-A = percentage of all words that contained 111 patterns; ramp = percentage of occurrence of three successive symbols which are increasing; RDI Relative dispersion index; S0 = singularity index; SamEn = sample entropy.
*While deeply sleeping.

Table 4
Summary of the main outcomes from this meta-analysis.

Emotional disorders	Physiological system	Measures proposed (as biomarkers)	Expected direction
Anxiety disorders	Brain & cardiac	LLE, D2, fractal dimension	Loss of complexity
	Respiratory	Entropy-related measures	Loss of ordered complexity (instability) for panic-disordered patients
Bipolar disorders*	Subjective & motor	spatial scaling exponent, DFA, Guzzetti's index	Loss of ordered complexity (instability) when a manic episode is upcoming or ongoing
Depressive disorders	Cardiac	DFA, entropy-related measures, fractal dimension	Loss of complexity
	Brain	DFA, fractal dimension, Lempel Ziv complexity index	Loss of ordered complexity (instability) in terms of integrated cerebral activity
	Endocrine	Entropy-related measures	Loss of ordered complexity (instability) for HPA hormones

Note. This table summarizes the main results expected when assessing emotional disorders considering nonlinear measures and taking into account the physiological system. Expected conclusions (directions) after assessment have been proposed according to results obtained in this meta-analysis.
HPA = hypothalamic-pituitary-adrenal axis.
Nonlinear measures: D2 = correlation dimension D2; DFA = detrended fluctuation analysis alpha exponents; LLE = largest Lyapunov exponent.
* Results are not conclusive for bipolar disorders.

2009; Yeragani et al., 2000, 2004a,b). Behavioural rigidity has also been pointed out by the deterministic structure of compulsive rituals in OCD patients (Bond and Guastello, 2013).

Conversely, informational measures showed different results to the extent that anxious participants exhibited higher information-generation rates in their physiological systems than the control subjects. It should be noticed, however, that this finding arises from studies that focused on the respiratory activity of panic patients (Caldirola et al., 2004; Yeragani et al., 2002a). It is well-established that these participants exhibit greater respiratory instability than the controls (Grassi et al., 2013; Niccolai et al., 2009). Respiratory instability has been linked to sympathetic hyperactivation and respiratory problems (Engoren, 1998; Garcia-Araujo et al., 2015; Yeragani et al., 2002a). This instability is not always related to high complexity and later healthier outcomes. Yang and Tsai (2013) stated that entropy-based methods may lead to confusing results because they do not allow ordered complexity to be distinguished from randomness, because both of them are characterised by high entropy values. These authors considered randomness to be the other extreme of mental disease and proposed some examples of pathological symptomatology with highly random-like patterns (confabulation, impulsiveness, etc.). In our opinion, the instability showed by panic-disordered respiratory systems may also be under the influence of random-like patterns due to deficits in control mechanisms involved in the regulation of interoceptive and exteroceptive inputs (Lehrer and Eddie, 2013). This proposition needs to be endorsed by empirical research with more sensitive measures (multiscale entropy or detrended fluctuation analysis, DFA, for instance) within the field of emotional disorders so as to distinguish ordered complexity from randomness (for further evidence in other populations, see Lai et al., 2010; Yang et al., 2015). Similar results can be obtained when panic-disordered patients' blood pressure is analysed, due to the influence of sympathetic components (Yeragani et al., 2004a,b).

The remaining studies with these measures are not in line with the aforementioned one, maybe due to the influence of medication on the cardiac system or the respiratory influence of panic-disordered patients (Baumert et al., 2009; Moon et al., 2013). However, scientific literature supports the fact that lower entropy may well appear among anxiety-disordered patients (discarding results from panic-disordered respiratory systems). In fact, some evidence stems from delivering treatments to ameliorate anxiety symptomatology or after inducing a sympathetic blockade (Bornas et al., 2007; Porta et al., 2013).

Regarding bipolar disorders, meta-analytic calculations fail to visualise significant overall effect sizes, mainly due to the wide heterogeneity amongst the studies. Mood fluctuations seem to show stochastic-like patterns and memory of initial conditions over time, as several studies have demonstrated in healthy people and also in bipolar participants (Bornas et al., 2015a; Gottschalk et al., 1995; Katerndahl and Wang, 2007; Ortiz et al., 2015; Young and Benton, 2015). However, mood may become more unpredictable when patients are under the influence of acute episodes, particularly manic ones, as shown by the activity monitored in different physiological systems (Bahrami et al., 2005; Bauer et al., 2011). Both complexity loss and the presence of random-like patterns of activity could cause the increases in unpredictability when a manic episode is coming on or ongoing, due to deficits in control mechanisms in regulating external and internal influences (Lehrer and Eddie, 2013; Yang and Tsai, 2013). Loss of complexity can be reflected in cardiac (higher sympathetic influence) and brain activity (increased delta-beta coupling) as a consequence of mood regulation and attention problems (Chen et al., 2008; Henry et al., 2010; Ozerdem et al., 2010). On the other hand, random-like patterns could be related to symptoms such as impulsive or risky behaviour which can be measured by monitoring frontal areas in the brain (increased delta synchronisation) and locomotor activity (Bahrami et al., 2005; Bullmore et al., 1994; Chen et al., 2008; Krane-Gartiser et al., 2014; Minassian et al., 2011).

Finally, depression has been the most studied disorder using nonlinear measures. One interesting finding is that results should be considered according to the physiological activity measured. Therefore, results arising from cardiac functioning show a loss of complexity in depressive patients reflected by less information generation over time, less patterns of variability in symbolic dynamics-related measures and higher values in measures within the scale invariant domain. Heart beating operates under the regulation of sympathetic and parasympathetic components. Better adjustment to contextual demands involves balanced variations in the dominant influence of one or another within the heart firing over time (West, 2006). Diminished parasympathetic control, mediated by the vagal nerve, has been strongly pointed to as a critical biomarker of depression, as well as of other diseases (Rottenberg, 2007; Porges, 2001; Shaffer et al., 2014). Parasym-

pathetic withdrawal has been linked to lower complexity (lower information and higher scale invariance) in patients with depressive disorders (Boettger et al., 2008; Voss et al., 2011; Yeragani et al., 2002b). Cardiac activity recordings while sleeping complement these findings and suggest that difficulty sleeping, as a symptom of depression, may have several links to low complexity and depression (Leistedt et al., 2011; Yang et al., 2011).

Conversely, findings arising from brain dynamics point to higher levels of information generation and lower values in scale invariance measures for patients with depression. In line with Lehrer and Eddie (2013), neuronal groups are working without sufficient inhibitory control being augmented and cancelled by each other in responding to the same tasks, leading to more complex, random firing patterns. This means that the activity arising from postsynaptic potentials and measured by EEG-based techniques is quantified as more complex, but shows more irregular and random-like behaviour. These findings are in agreement with some theories that endorse the existence of deficits in inhibition mechanisms in depressive patients from molecular mechanisms and heightened desynchronisation/disconnection in the brain circuits of these patients (Croarkin et al., 2011; Fingelkurts et al., 2007; Olbrich et al., 2014; Takahashi, 2013). Detrended fluctuation analysis (DFA) complements these arguments providing some evidence in favour of the existence of random-like patterns in brain dynamics (Leistedt et al., 2007). Linkenkaer-Hansen et al. (2005) found that depressed patients showed DFA exponents on the verge of 0.50 (i.e. uncorrelated data; see Peng et al., 1995) when theta band activity in temporocentral areas were considered. This was attributed to neuronal hyperactivity caused by limbic-cortical impairments.

Results from the nonlinear analyses of endocrine activity also showed an increased irregularity when stress hormones were considered, mainly explained by a hyperactivation of adrenal gonads via sympathetic influences and deficits in inhibitory control mechanisms (Carroll et al., 2012; Pariante and Lightman, 2008; Posener et al., 2004).

None of the other factors included in the study (gender, age, methodological quality, etc) moderated the discriminative role of nonlinear measures, as conducted meta-regressions showed. The possible explanation for this could be the lack of variability in the samples, that is, the large majority of these studies consisted of adult subjects with a relatively equal proportion of members from both sexes and with equivalent methodological quality, amongst others.

4.1. Limitations of this study

A limitation of this study is that it failed to extract conclusions from three of the studied categories of nonlinear measures (statistical, geometric, and energetic measures) and, in some cases, it was impossible to conduct ANOVA-based analyses, or other explanatory analyses, for all of the studied branches of disorders. This is due to the low sample sizes we found which hindered the possibility of conducting meta-analytic statistics. Further research should be carried out in order to complement the findings obtained in this study. Moreover, publication biases were detected in some of our results, highlighting that those findings should treated with caution. In our favour, it is worth mentioning the exhaustiveness of our search procedure locating and reviewing more than 5000 papers taking into account how recent the application of NDS in Psychology is (see Fig. 1). Low sample sizes in some of the analysed studies may therefore explain these results. In this regard, it is highly recommended that larger sample sizes are recruited in further studies. Finally, it also is important to suggest that more studies, with more precise measures, should be undertaken in order to enhance the accuracy of the conclusions reached in them for psychopathology, as some articles have already shown (Bornas et al., 2006, 2015b). Likewise, further research should be included in order to extract conclusions from disease severity and specific subtypes for each branch of disorders.

4.2. Complexity, ordered complexity, and optimum variability. Suggestions for further research

Now we should return to, and focus on, the elusive concept of complexity (see Burggren and Monticino, 2005; for a review) and the general hypothesis that complexity losses characterise diseased systems. In this meta-analysis we have noted that many different metrics have been used to evaluate the complexity of diseased and non-diseased systems. According to the classification introduced by Bravi et al. (2011) these metrics belong to several variability domains (e.g. approximate entropy belongs to the informational domain) and we have followed this classification. It should be noticed that some measures (e.g. approximate entropy or largest Lyapunov exponent) capture deterministic variability and dependency between points in a time series whereas others do not do so (e.g. standard deviations in the statistical domain). Researchers correctly interpret the values they have obtained using any of these metrics as increases or decreases in the complexity of the system they are working on. However, when several studies are looked at together, such as in a meta-analytic study, and different metrics have been used, complexity increases and decreases have to be clearly separated to compute, for example, overall effect sizes. For instance, the increases in the entropy of the respiratory signals from panic disordered patients (Caldirola et al., 2004) might be interpreted as increases in the complexity of that system, much as decreases in the entropy of the cardiovascular system of depressed patients (Moon et al., 2013) are interpreted as decreases in the complexity of the patients' cardiac systems (see Vargas et al., 2015). Nevertheless, we know that simple systems can exhibit random behaviour, so that if we calculate the entropy of that system's behaviour over a period of time we will get a high entropy value that cannot be understood to reflect the behaviour of a complex system (Yang and Tsai, 2013). To distinguish complexity from randomness, and to clarify the search for nonlinear biomarkers of psychological diseases, we can consider the loss of *ordered complexity* instead of the loss of (any) complexity. In doing so, the aforementioned entropy increases in panic-disordered patients can be interpreted as ordered complexity decreases instead of complexity increases.

Therefore, a loss of complexity as a biomarker of emotional disorder could be reformulated as a *loss of ordered complexity*. This change, however, does not solve the problem associated with the word "loss", which in general means "decrease" or "subtraction", and therefore interpreting increases in specific measures like sample entropy, or symbolic dynamics such as complexity losses, remains difficult as it looks like a word game. Fortunately, there is a better approach to this problem: *optimum variability* (see Schuldberg, 2015; for a recent review). Guastello (2015) notices that complex systems generally display mid-range values of variability/complexity and that both low-range values and high-range values could be maladaptive. Therefore, nonlinear biomarkers of disease should not be looked at as complexity decreases or losses, but as deviations (increases and decreases) from the optimum variability of the system under study. Ideally, future research should be aimed at identifying the optimum variability range of values for each biomarker. For instance, leading questions could be asked such as, what range of entropy values the optimum variability of the panic disordered patients' respiratory system consist of?, or, what range of fractal dimension values does the optimum variability of the PTSD patients' EEG theta-band dynamics consist of? One important question to be addressed in future studies concerns the clinical usefulness of nonlinear biomarkers in order to detect abnormalities before the onset of an emotional disorder. The studies reviewed

in the current meta-analysis worked on diagnosed patients, and therefore we cannot discern if the biomarkers were present before patients were clinically diagnosed. In other words, it remains to be answered whether these markers are state or trait dependent. If they were trait dependent then they would reflect a vulnerability feature with important implications for the prevention of those disorders.

The large number of different nonlinear measures should reduce as research for nonlinear biomarkers advances and grows. This research is just beginning in historical terms, but ground-breaking in psychological science, and it is therefore understandable that many signs are evaluated as possible candidates for biomarkers of specific diseases. It seems reasonable, however, to expect that inasmuch as some candidates receive empirical support and consolidate its "biomarker" character, other less supported candidates will be removed from the long list we currently have. Narrowing down this list would make it easier to specify the optimum variability range for each biomarker. However, it is worth noting to take into account the results obtained due to the robust methodological approach for reviewing scientific literature that we followed.

To sum up, emotional disorders definitely constitute the most common targets in terms of mental health. This meta-analysis has revealed that the search for nonlinear biomarkers of these disorders has been fruitful but, as in any growing field, many controversial issues emerged that should be addressed in the near future. It seems quite clear, however, that the NDS theory is the appropriate framework for this search for biomarkers and, therefore, it will help to develop more precise diagnostic methods and more effective treatments for emotional disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neubiorev.2016.05.023.

References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. American Psychiatric Association, Washington, DC.
Bahrami, B., Seyedsadjadi, R., Babadi, B., Noroozian, M., 2005. Brain complexity increases in mania. Neuroreport 16, 187–191.
Bak, P., Tang, C., Wiesenfeld, K., 1987. Self-organized criticality: an explanation of the 1/f noise. Phys. Rev. Lett., 59, http://dx.doi.org/10.1103/PhysRevLett.59.381.
Bauer, M., Glenn, T., Alda, M., Grof, P., Sagduyu, K., Bauer, R., et al., 2011. Comparison of pre-episode and pre-remission states using mood ratings from patients with bipolar disorder. Pharmacopsychiatry 44, S49–S53, http://dx.doi.org/10.1055/s-0031-1273765.
Baumert, M., Lambert, G.W., Dawood, T., Lambert, E.A., Esler, M.D., McGrane, M., Nalivaiko, E., 2009. Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder. Am. J. Physiol. Heart Circ. Physiol. 297, H649–H674, http://dx.doi.org/10.1152/ajpheart.00236.2009.
Bob, P., Kukleta, M., Riecansky, I., Susta, M., Kukumberg, P., Jagla, F., 2006. Chaotic EEG patterns during recall of stressful memory related to panic attack. Physiol. Res. 55, S113–S119.
Boettger, S., Hoyer, D., Falkenhahn, K., Kaatz, M., Yeragani, V.K., Bar, K.-J., 2008. Nonlinear broad band dynamics are less complex in major depression. Bipolar Disord. 10, 276–284, http://dx.doi.org/10.1111/j.1399-5618.2007.00503.x.
Bond Jr., R.W., Guastello, S.J., 2013. Aperiodic deterministic structure of OCD and the familial effect on rituals. Nonlinear Dyn. Psychol. Life Sci. 17, 465–491.
Bornas, X., Llabres, J., Noguera, M., Lopez, A.M., Gelabert, J.M., Vila, I., 2006. Fear induced complexity loss in the electrocardiogram of flight phobics: a multiscale entropy analysis. Biol. Psychol. 73, 272–279.
Bornas, X., Llabres, J., Tortella-Feliu, M., Fullana, M.A., Montoya, P., Lopez, A.M., et al., 2007. Vagally mediated heart rate variability and heart rate entropy as predictors of treatment outcome in flight phobia. Biol. Psychol. 76, 188–195.
Bornas, X., Balle, M., Morillas-Romero, A., Aguayo-Siquier, B., 2015a. Allometric control of daily mood and anxiety fluctuations. Motiv. Emot. 39, 571–579, http://dx.doi.org/10.1007/s11031-015-9471-4.
Bornas, X., Balle, M., de la Torre-Luque, A., Fiol-Veny, A., Llabres, J., 2015b. Ecological assessment of heart rate complexity: differences between high- and low-anxious adolescents. Int. J. Psychophysiol. 98, 112–118, http://dx.doi.org/10.1016/j.ijpsycho.2015.07.007.
Botella, J., Gambara, H., 2002. Qué es el meta-análisis [What is Meta-Analysis]. Biblioteca Nueva, Madrid.
Bravi, A., Longtin, A., Seely, A.J.E., 2011. Review and classification of variability analysis techniques with clinical applications. Biomed. Eng. Online 10, http://dx.doi.org/10.1186/1475-925X-10-90.
Bullmore, E., Brammer, M., Harvey, I., Persaud, R., Murray, R., Ron, M., 1994. Fractal analysis of the boundary between white matter and cerebral cortex in magnetic resonance images: a controlled study of schizophrenic and manic-depressive patients. Psychol. Med. 24, 771–781.
Burggren, W.W., Monticino, M.G., 2005. Assessing physiological complexity. J. Exp. Biol. 208, 3221–3232, http://dx.doi.org/10.1242/jeb.01762.
Caldirola, D., Bellodi, L., Caumo, A., Migliarese, G., Perna, G., 2004. Approximate entropy of respiratory patterns in panic disorder. Am. J. Psychiatry 161, 79–87.
Carroll, B.J., Iranmanesh, A., Keenan, D.M., Cassidy, F., Wilson, W.H., Veldhuis, J.D., 2012. Pathophysiology of hypercortisolism in depression: pituitary and adrenal responses to low glucocorticoid feedback. Acta Psychiatr. Scand. 125, 478–491, http://dx.doi.org/10.1111/j.1600-0447.2011.01821.x.
Chae, J.-H., Jeong, J., Peterson, B.S., Kim, D.-J., Bahk, W.-M., Jun, T.-Y., et al., 2004. Dimensional complexity of the EEG in patients with posttraumatic stress disorder. Psychiatry Res. Neuroimaging 131, 79–89, http://dx.doi.org/10.1016/j.pscychresns.2003.05.002.
Chen, S.-S., Tu, P.-C., Su, T.-P., Hsieh, J.-C., Lin, Y.-C., Chen, L.-F., 2008. Impaired frontal synchronization of spontaneous magnetoencephalographic activity in patients with bipolar disorder. Neurosci. Lett. 445, 174–178, http://dx.doi.org/10.1016/j.neulet.2008.08.080.
Chialvo, D.R., 2010. Emergent complex neural dynamics. Nat. Phys. 6, 744–750, http://dx.doi.org/10.1038/nphys1803.
Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Lawrence Erlbaum, Mahwah.
Costa, M., Goldberger, A.L., Peng, C.K., 2002. Multiscale entropy analysis of complex physiologic time series. Phys. Rev. Lett. 89, 068102.
Croarkin, P.E., Levinson, A.J., Daskalakis, Z.J., 2011. Evidence for GABAergic inhibitory deficits in major depressive disorder. Neurosci. Biobehav. Rev. 35, 818–825, http://dx.doi.org/10.1016/j.neubiorev.2010.10.002.
De Graaf, R., ten Have, M., van Gool, C., van Dorssalaer, S., 2012. Prevalence of mental disorders and trends from 1996 to 2009. Results from The Netherlands Mental Health Survey and Incidence Study-2. Soc. Psychiatry Psychiatr. Epidemiol. 47, 203–213, http://dx.doi.org/10.1007/s00127-010-0334-8.
Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple graphical test. BMJ 315, 629–634.
Engoren, M., 1998. Approximate entropy of respiratory rate and tidal volume during weaning from mechanical ventilation. Crit. Care Med. 26, 1817–1823.
Evans, D., 2003. Hierarchy of evidence: a framework for ranking evidence evaluating healthcare interventions. J. Clin. Nurs. 12, 77–84, http://dx.doi.org/10.1046/j.1365-2702.2003.00662.x.
Ferguson, C.J., Brannick, M.T., 2012. Publication bias in psychological science: prevalence, methods for identifying and controlling, and implications for the use of meta-analyses. Psychol. Methods 17, 120–128, http://dx.doi.org/10.1037/a0024445.
Fingelkurts, A.A., Fingelkurts, A.A., Rytsala, H., Suominen, K., Isometsa, E., Kahkonen, S., 2007. Impaired functional connectivity at EEG alpha and theta frequency bands in major depression. Hum. Brain Mapp. 28, 247–261.
Freeman, W.J., 1999. How Brains Make up Their Minds. Weidenfeld & Nicolson, Berkeley.
Friedman, B.H., 2007. An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. Biol. Psychol. 74, 185–199.
Garcia-Araujo, A.S., di Lorenzo, P., Labadessa, I.G., Jurgensen, S.P., di Thomazo-Luporini, L., et al., 2015. Increased sympathetic modulation and decreased response of the heart rate variability in controlled asthma. J. Asthma 52, 246–253, http://dx.doi.org/10.3109/02770903.2014.957765.
Garrett, D.D., Samanez-Larkin, G.R., MacDonald, S.W.S., Lindenberger, U., McIntosh, A.R., Grady, C.L., 2013. Moment-to-moment brain signal variability: a next frontier in human brain mapping? Neurosci. Biobehav. Rev. 37, 610–624, http://dx.doi.org/10.1016/j.neubiorev.2013.02.015.
Goldberger, A.L., Amaral, L.A., Hausdorff, J.M., Ivanov, P.C., Peng, C.K., Stanley, H.E., 2002. Fractal dynamics in physiology: alterations with disease and aging. Proc. Natl. Acad. Sci. U. S. A. 19, 2466–2472.
Goldberger, A.L., 1996. Biomedical Oscillations and Cellular Rhythms: the Molecular Basis of Periodic and Chaotic Behavior. Cambridge University Press, Cambridge.

Gottschalk, A., Bauer, M.S., Whybrow, P.C., 1995. Evidence of chaotic mood variation in bipolar disorder. Arch. Gen. Psychiatry 52, 947–959.
Grassi, M., Caldirola, D., Vanni, G., Guerreiro, G., Piccinni, M., Valchera, A., et al., 2013. Baseline respiratory parameters in panic disorder: a meta-analysis. J. Affect. Disord. 146, 173–185, http://dx.doi.org/10.1016/j.jad.2012.08.034.
Guastello, S.J., Koopmans, M., Pincus, D. (Eds.), 2009. Cambridge University Press, New York.
Guastello, S.J., 2004. Progress in applied nonlinear dynamics: welcome to NDPLS. Nonlinear Dyn. Psychol. Life Sci. 8, 1–15.
Guastello, S.J., 2015. The complexity of the psychological self and the principle of optimum variability. Nonlinear Dyn. Psychol. Life Sci. 19, 511–528.
Hedges, L.V., Olkin, I., 1985. Statistical Methods for Meta-analysis. Academic Press, Orlando.
Hedges, L.V., Pigott, T.D., 2004. The power of statistical tests for moderators in metaanalysis. Psychol. Methods 9, 426–445.
Henry, B.L., Minassian, A., Paulus, M.P., Geyer, M.A., Perry, W., 2010. Heart rate variability in bipolar mania and schizophrenia. J. Psychiatr. Res. 44, 168, http://dx.doi.org/10.1016/j.jpsychires.2009.07.011.
Higgins, J.P.T., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558.
Katerndahl, D.A., Wang, C.-P., 2007. Dynamic covariation of symptoms of anxiety and depression among newly-diagnosed patients with major depressive episode, panic disorder, and controls. Nonlinear Dyn. Psychol. Life Sci. 11, 349–366.
Krane-Gartiser, K., Henriksen, T.E.G., Morken, G., Vaaler, A., Fasmer, B., 2014. Actigraphic assessment of motor activity in acutely admitted inpatients with bipolar disorder. PLoS One 9, e89574, http://dx.doi.org/10.1371/journal.pone.0089574.
Lai, M.C., Lombardo, M.V., Chakrabarti, B., Sadek, S.A., Pasco, G., Wheelwright, S.J., et al., 2010. A shift to randomness of brain oscillations in people with autism. Biol. Psychiatry 68, 1092–1099, http://dx.doi.org/10.1016/j.biopsych.2010.06.027.
Lehrer, P., Eddie, D., 2013. Dynamic processes in regulation and some implications for biofeedback and biobehavioral interventions. Appl. Psychophysiol. Biofeedback 38, 143–155, http://dx.doi.org/10.1007/s10484-013-9217-6.
Leistedt, S.J.-J., Dumont, M., Lanquart, J.-P., Jurysta, F., Linkowski, P., 2007. Characterization of the sleep EEG in acutely depressed men using detrended fluctuation analysis. Clin. Neurophysiol. 118, 940–950, http://dx.doi.org/10.1016/j.clinph.2007.01.003.
Leistedt, S.J.-J., Linkowski, P., Lanquart, J.-P., Mietus, J.E., Davis, R.B., Goldberger, A.L., Costa, M.D., 2011. Decreased neuroautonomic complexity in men during an acute major depressive episode: analysis of heart rate dynamics. Transl. Psychiatry 1, e27, http://dx.doi.org/10.1038/tp.2011.23.
Linkenkaer-Hansen, K., Monto, S., Rytsala, H., Suominen, K., Isometsa, E., Kahkonen, S., 2005. Breakdown of long-range temporal correlations in theta oscillations in patients with major depressive disorder. J. Neurosci. 25, 10131–10137, http://dx.doi.org/10.1523/jneurosci.3244-05.2005.
Lipsey, M.W., Wilson, D.B., 2001. Practical Meta-analysis. Sage Publications Inc., Thousand Oaks.
Mandelbrot, B.B., 1967. How long is the Coast of Britain? Statistical self-similarity and fractal dimension. Science 156, 636–640.
Martin, E.I., Ressler, K.J., Binder, E., Nemeroff, C.B., 2009. The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. Psychiatr. Clin. North Am. 32, 549–575, http://dx.doi.org/10.1016/j.psc.2009.05.004.
Minassian, A., Henry, B.L., Young, J.W., Masten, V., Geyer, M.A., Perry, W., 2011. Repeated assessment of exploration and novelty seeking in the human behavioral pattern monitor in bipolar disorder patients and healthy individuals. PLoS One 6, e24185, http://dx.doi.org/10.1371/journal.pone.0024185.
Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev., 4, http://dx.doi.org/10.1186/2046-4053-4-1.
Moon, E., Lee, S.-H., Kim, D.-H., Hwang, B., 2013. Comparative study of heart rate variability in patients with schizophrenia, bipolar disorder, post-traumatic stress disorder, or major depressive disorder. Clin. Psychopharmacol. Neurosci. 11, 137–143, http://dx.doi.org/10.9758/cpn.2013.11.3.137.
Niccolai, V., van Duinen, M.A., Griez, E.J., 2009. Respiratory patterns in panic disorder reviewed: a focus on biological challenge tests. Acta Psychiatr. Scand. 120, 167–177, http://dx.doi.org/10.1111/j.1600-0447.2009.01408.x.
Olbrich, S., Traenkner, A., Chittka, T., Hegerl, U., Schoenknecht, P., 2014. Functional connectivity in major depression: increased phase synchronization between frontal cortical EEG-source estimates. Psychiatry Res. 222, 91–99, http://dx.doi.org/10.1016/j.pscychresns.2014.02.010.
Ortiz, A., Bradler, K., Garnham, J., Slaney, C., Alda, M., 2015. Nonlinear dynamics of mood regulation in bipolar disorder. Bipolar Disord. 17, 139–149, http://dx.doi.org/10.1111/bdi.12246.
Ozerdem, A., Guntekin, B., Saatci, E., Tunca, Z., Basar, E., 2010. Disturbance in long distance gamma coherence in bipolar disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 861–865, http://dx.doi.org/10.1016/j.pnpbp.2010.04.001.
Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31, 464–468, http://dx.doi.org/10.1016/j.tins.2008.06.006.
Peng, C.K., Havlin, S., Stanley, H.E., Goldberger, A.L., 1995. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos 5, 82–87.
Pikkujamsa, S.M., Makikallio, T.H., Sourander, L.B., Raiha, I.J., Puukka, P., Skytta, J., et al., 1999. Cardiac interbeat interval dynamics from childhood to senescence: comparison of conventional and new measures based on fractals and chaos theory. Circulation 100, 393–399.
Pincus, S.M., 1995. Approximate entropy (ApEn) as a complexity measure. Chaos 5, 110–117.
Pittman-Poletta, B.R., Scheer, F.A., Butler, M.P., Shea, S.A., Hu, K., 2013. The role of the circadian system in fractal neurophysiological control. Biol. Rev. Camb. Philos. Soc. 88, 873–894, http://dx.doi.org/10.1111/brv.12032.
Porges, S.W., 2001. The polyvagal theory: phylogenetic substrates of a social nervous system. Int. J. Psychophysiol. 42, 123–146.
Porta, A., Castiglioni, P., di Rienzo, M., Bari, V., Bassani, T., Marchi, A., et al., 2013. Information domain analysis of the spontaneous baroreflex during pharmacological challenges. Auton. Neurosci. Basic Clin. 178, 67–75, http://dx.doi.org/10.1016/j.autneu.2013.03.003.
Posener, J.A., DeBattista, C., Veldhuis, J.D., Province, M.A., Williams, G.H., Schatzberg, A.F., 2004. Process irregularity of cortisol and adrenocorticotropin secretion in men with major depressive disorder. Psychoneuroendocrinology 29, 1129–1137, http://dx.doi.org/10.1016/j.psyneuen.2004.01.004.
Rice, M.J., 2008. Evidence-based practice in psychiatric and mental health nursing: qualitative meta-synthesis. J. Am. Psychiatr. Nurses Assoc. 14, 382–385, http://dx.doi.org/10.1177/1078390308326661.
Richman, J.A., Moorman, J.R., 2000. Physiological time-series analysis using approximate entropy and sample entropy. Am. J. Physiol. Heart Circ. Physiol. 278, H2039–H2049.
Rottenberg, J., 2007. Cardiac vagal control in depression: a critical analysis. Biol. Psychol. 74, 200–211, http://dx.doi.org/10.1016/j.biopsycho.2005.08.010.
Schuldberg, D., 2015. What is optimum variability? Nonlinear Dyn. Psychol. Life Sci. 19, 553–568.
Shaffer, F., McCratty, R., Zerr, C.L., 2014. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front. Psychol. 5, 1040, http://dx.doi.org/10.3389/fpsyg.2014.01040.
Stiedl, O., Jansen, R.F., Pieneman, A.W., Ogren, S.O., Meyer, M., 2009. Assessing aversive emotional states through the heart in mice: implications for cardiovascular dysregulation in affective disorders. Neurosci. Biobehav. Rev. 33, 181–190, http://dx.doi.org/10.1016/j.neubiorev.2008.08.015.
Takahashi, T., 2013. Complexity of spontaneous brain activity in mental disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 45, 258–266, http://dx.doi.org/10.1016/j.pnpbp.2012.05.001.
Thayer, J.F., Lane, R.D., 2009. Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. Neurosci. Biobehav. Rev. 33, 81–88, http://dx.doi.org/10.1016/j.neubiorev.2008.08.004.
Tonhajzerova, I., Ondrejka, I., Chladekova, L., Farsky, I., Visnovcova, Z., Calkovska, A., Javorka, M., 2012. Heart rate time irreversibility is impaired in adolescent major depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 39, 212–217, http://dx.doi.org/10.1016/j.pnpbp.2012.06.023.
Vargas, B., Cuesta-Frau, D., Ruis-Esteban, R., Cirugeda, E., Varela, M., 2015. What the biosignal entropy tell us about health and disease? Applications in some clinical fields. Nonlinear Dyn. Psychol. Life Sci. 19, 419–436.
Voss, A., Boettger, M.K., Schulz, S., Gross, K., Bar, K.-J., 2011. Gender-dependent impact of major depression on autonomic cardiovascular modulation. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1131–1138, http://dx.doi.org/10.1016/j.pnpbp.2011.03.015.
Wells, G.A., Shea, B., O'Connell, D., Petersen, J., Welch, V., Losos, M., Tugwell, P., 2014. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomized Studies in Meta-analyses. Department of Epidemiology and Community Medicine, University of Ottawa, Canada.
Werner, G., 2010. Fractals in the nervous system: conceptual implications for theoretical neuroscience. Front. Physiol. 1, 15, http://dx.doi.org/10.3389/fphys.2010.00015.
West, B.J., 1990. Fractal Physiology and Chaos in Medicine. World Scientific Publishing, Singapore.
West, B.J., 2006. Where Medicine Went Wrong: Rediscovering the Path to Complexity. World Scientific Publishing, Singapore.
Yang, A.C., Tsai, S.-J., 2013. Is mental illness complex? From behavior to brain. Prog. Neuro-Psychopharmacol. Biol. Res. 45, 253–257, http://dx.doi.org/10.1016/j.pnpbp.2012.09.015.
Yang, A.C., Tsai, S.-J., Yang, C.H., Kuo, C.-H., Chen, T.-J., Hong, C.-J., 2011. Reduced physiologic complexity is associated with poor sleep in patients with major depression and primary insomnia. J. Affect. Disord. 131, 179–185, http://dx.doi.org/10.1016/j.jad.2010.11.030.
Yang, A.C., Hong, C.-J., Liou, Y.-J., Huang, K.-L., Huang, C.-C., Liu, M.-E., et al., 2015. Decreased resting-state brain activity complexity in schizophrenia characterized by both increased regularity and randomness. Hum. Brain Mapp. 36, 2174–2186, http://dx.doi.org/10.1002/hbm.22763.
Yeragani, V.K., Nadella, R., Hinze, B., Yeragani, S., Jampala, V.C., 2000. Nonlinear measures of heart period variability: decreased measures of symbolic dynamics in patients with panic disorder. Depression Anxiety 12, 67–77.
Yeragani, V.K., Radhakrishna, R.K.A., Tancer, M., Uhde, T., 2002a. Nonlinear measures of respiration: respiration irregularity and increased chaos of respiration in patients with panic disorder. Biol. Psychiatry 46, 111–120, http://dx.doi.org/10.1159/000066388.

Yeragani, V.K., Rao, R.K., Smitha, M.R., Pohl, R.B., Balon, R., Srinivasan, K., 2002b. Diminished chaos of heart rate time series in patients with major depression. Biol. Psychiatry 51, 733–744.
Yeragani, V.K., Mallavarapu, M., Radhakrishna, R.K.A., Tancer, M., Uhde, T., 2004a. Linear and nonlinear measures of blood pressure variability: increased chaos of blood pressure time series in patients with panic disorder. Depression Anxiety 19, 85–95.
Yeragani, V.K., Radhakrishna, R.K.A., Ramakrishnan, K.R., Srinivasan, S.H., 2004b. Measures of LLE of heart rate in different frequency bands: a possible measure of relative vagal and sympathetic activity. Nonlinear Anal. Real World Appl. 5, 441–462, http://dx.doi.org/10.1016/j.nonrwa.2003.07.002.
Young, H., Benton, D., 2015. We should be using nonlinear indices when relating heart-rate dynamics to cognition and mood. Sci. Rep. 5, 16619, http://dx.doi.org/10.1038/srep16619.

Anxiety from early to middle adolescence: Developmental trajectories and associations with temperamental factors

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Abstract The transition from early to middle adolescence represents a sensitive period for the expression of anxiety symptomatology. Accordingly, anxiety symptoms may emerge or be exacerbated during this time. Many factors may influence the course of anxiety over this period and put individuals at risk for a full−blown disorder. Among these factors, gender and temperament must be stressed. This study aimed to (a) describe how anxiety evolves throughout the developmental period from early to middle adolescence, (b) identify varying trajectories of anxiety symptoms over this transition, and (c) characterize the anxiety trajectories in function of gender and temperamental risk factors so as to identify the profile of adolescents at risk for an anxiety disorder. A sample of 884 adolescents (44.40% boys; mean initial age = 13.01, *SD* = 0.56) were monitored over an 18−month period and assessed every six months. Anxiety and three constructs related to fearful temperament and attentional control were measured. Growth mixture modeling was used to examine the latent general course and individual−specific course of anxious symptomatology. A decreasing, curvilinear course of anxiety symptoms over the assessment period was revealed. Moreover, varying trajectories were identified for all subtypes of anxiety symptomatology, varying from three to six trajectory classes. Finally, classes including at−risk adolescents with elevated levels of symptoms across assessments were characterized by high fearful temperament, low attention control, and being female. To sum up, anxiety symptomatology tends to decrease from early to middle adolescence, but especial attention must be paid to temperament and gender.

Keywords: Anxiety symptoms, development, temperament, adolescence, growth.

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Adolescent anxiety disorders (ADs) may have a dramatic impact on adjustment and may become a trigger for severe mental disorders in adulthood (Balasz et al., 2013; Beesdo−Baum & Knappe, 2012; Essau, Lewinsohn, Olaya, & Seeley, 2014). Studies on developmental psychopathology have focused on depicting how anxious symptomatology evolves during adolescence (e.g., Betts et al., 2016; Leadbeater, Thompson, & Gruppuso, 2012; Nelemans et al., 2014; Van Oort, Greaves−Lord, Ormel, Verhulst, & Huizink, 2011). Findings from these studies are quite inconsistent: some point to a smooth curvilinear decrease of anxiety throughout adolescence, while others suggest that it follows a more linear or even increasing course. These discrepancies could be due to the different lengths of the study periods (usually all of adolescence, but some of them even include long periods of child-

hood or adulthood), or to the timing of assessments (annually or biannually).

Along with these longitudinal studies covering developmental periods over several years, there is a need to focus on shorter periods of time, and in particular the period from early to middle adolescence, as it is considered to be a sensitive period for the expression of concrete anxiety symptoms. According to Weems (2008), differential symptomatology may be expressed as adolescence progresses (e.g., separation anxiety symptoms are more common in early adolescence, panic in late adolescence, etc.). In this regard, a higher predominance of social phobia symptomatology is observed during the period from early to middle adolescence as well as higher rates of full−blown social phobia diagnosed (Beesdo−Baum & Knappe, 2012; Copeland, Angold, Shanahan, & Costello, 2014; Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). On the other hand, assessment timing is a critical issue when examining the developmental course of ADs because maturational processes may work at relatively shorter time scales in adolescence (even monthly) and relevant developmental changes may go unnoticed if the consecutive assessment points are scheduled too far apart.

Also, the individual−specific course of adolescent anxiety development is scarcely considered. Quoting Weems (2008), " a functional model of continuity and change in anxious emotion must also take into consideration the reality that ideographic or individually experienced factors will also shape the expression of anxious emotion" (p. 494). In recent years, this aspect is receiving increasing attention, and several studies have highlighted the presence of different relatively heterogeneous courses of AD symptomatology throughout adolescence (Letcher, Sanson, Smart, & Toumbourou, 2012; Nelemans et al., 2014; Olino, Klein, Lewinsohn, Rohde, & Seeley, 2010). Current methods for modeling longitudinal data allow for the trajectories of specific outcomes (anxiety symptom developmental courses) to be depicted and thus the identification of distinct clusters of individuals with similar developmental attributes. From there, individuals who may develop courses of maladaptive AD symptomatology (at−risk courses) can be identified.

The link between person−centered factors and anxiety development course of ADs because maturational processes may work at relatively shorter time scales in adolescence (even monthly) and relevant developmental changes may go unnoticed if the consecutive assessment points are scheduled too far apart.

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The link between person−centered factors and anxiety development

In addition to maturation, the development of anxiety may depend on other factors (see Beesdo−Baum & Knappe, 2012). The most studied factor has been gender (e.g., Nelemans et al., 2014; Van Oort, Greaves−Lord, Verhulst, Ormel, & Huizink, 2009) and results have suggested that girls show higher anxious symptomatology in general.

Besides gender, temperamental characteristics, considered to be a precursor of adult personality traits, may have a strong influence on anxiety throughout adolescence (Bijttebier, Beck, Claes, &

Vandereycken, 2009; De Pauw & Mervielde, 2010; Tortella−Feliu, Balle, & Sese, 2010; Van Oort et al., 2011). Some temperamental traits have been identified as risk factors for the development of AD, too. In this regard, a specific component of regulatory temperament called attentional control (AC) merits study. AC has been clearly linked with the development of ADs, taking the focus on the lack of disengagement from anxiogenic stimuli and thereby appears to be strongly associated with high AD symptomatology (Sportel, Nauta, de Hullu, de Jong, & Hartman, 2011; Susa, Pitica, Benga, & Miclea, 2012).

On the other hand, it is important to mention fearful temperament (FT). FT comprises varying traits that account for reactive temperament and make individuals likely to feel defensive emotions (e.g., fear) when faced with new or unfamiliar situations and to avoid potentially threatening stimuli (see Rapee & Coplan, 2010). In this study, we put the spotlight on three concrete FT constructs. The first one is sensitivity to punishment (SP), which is responsible for organizing behavioral responses toward inhibition and avoidance of high−intensity stimuli and new or potentially threatening events (Bijttebier et al., 2009; Gray, 1982; Gray & McNaughton, 2000). The second construct is negative affectivity (NA)−this is strongly related to neuroticism−which is defined as the expression of an unpleasant affect as a consequence of confronting potentially aversive stimuli (Capaldi & Rothbart, 1992; Rothbart, 2007). Finally, we consider shyness, a more specific social trait that has also been associated with anxious symptomatology, especially social phobia (Karevold, Ystrom, Coplan, Sanson, & Mathiesen, 2012; Rubin, Coplan, Bowker, & Menzer, 2011).

Temperamental factors must be considered dynamically throughout adolescence due to the nature of maturation towards adult personalities. Changes in these temperamental factors have been linked to fluctuations in anxious symptomatology and emotion regulation (Laceulle, Nederhof, Karreman, Ormel, & van Aken, 2012; Laceulle, Ormel, Vollerbergh, van Aken, & Nederhof, 2014).

The present study

The study of the trajectories of anxious symptomatology along with the influence of temperamental factors should lead to the identification of risk profiles in individuals who develop an AD during or after adolescence. This is particularly important in periods as critical to the development of ADs as the period from early to middle adolescence is, considered as a sensitive period for social anxiety. This study aimed to examine the trajectory of both overall anxiety and specific AD symptomatology (e.g., panic disorder, social phobia, separation anxiety, obsessive compulsive anxiety, and generalized anxiety) over the period from early to middle adolescence. Anxiety scores were taken at 6−month intervals for 1.5 years. We expected a curvilinear course of anxious symptomatology along the assessment points, with a high level of social phobia symptoms across assessments.

The second aim of the study was to examine the varying trajectories of AD symptomatology throughout our assessment period. We expected to find at least two different trajectories for each type of symptomatology in order to identify the developmental course of individuals who might be at risk for developing an AD (those with sustained high levels or clinical levels of anxiety at all assessment points).

Finally, we aimed to identify different courses of AD symptomatology among adolescents with the differential influence of gender and temperamental factors taken into account. In this regard, we hypothesized that individuals at risk for ADs would show a developmental course of anxiety symptomatology that was influenced by the aforementioned temperamental factors and gender (concretely, being girl, and showing low AC and high SP and NA across assessments). In the case of social phobia, shyness would play a significant role in the at−risk class; and SP and NA, as more general temperamental factors, would influence all of the AD−specific symptoms.

Methods

Participants

A sample of 934 Spanish adolescents (45.40% boys; 13.01 years old on average at the first assessment point, $SD = 0.56$) participated in this study. All of them were Caucasians. They were recruited from state schools and came from middle−class families. Participants were recruited from the first level of secondary education. All of the participants were able to write, read, and speak fluently in both Spanish and Catalan, and each handed in a written consent form signed by themselves and their legal guardians. None of the participants showed severe disabilities, mental retardation, or neurological disorders according to parents and school board reports.

Psychological Instruments

Anxiety symptomatology. The Revised Child Anxiety and Depression Scale (RCADS; Chorpita, Yim, Moffit, Umemoto, & Francis, 2000) consists of 47 self−report items on a 4−point response scale. This questionnaire evaluates symptomatologies of five different ADs (separation anxiety disorder, social phobia, generalized anxiety disorder, panic disorder, and obsessive compulsive disorder) and allows for an index of total anxiety symptomatology to be calculated. Reliability levels were satisfactory across assessment points (Cronbach′s alpha from .67 to .87 for the subscales, and from .92 to .94 for the total anxiety scale). Correlations between points of measurement ranged from Pearson′s $r = .31$ to $r = .75$ (all $p < .05$), for all the scales across the four sample points in our study.

Temperamental factors. The Early Adolescence Temperament Questionnaire (EATQ−Revised long form; Ellis & Rothbart, 2001) is comprised of 103 items on a 5−point Likert scale. The EATQ−R allows for 13 different domains and four principal temperamental factors (negative affectivity [NA], effortful control, affiliativeness, and surgency; Rothbart, 2007) to be assessed. Due to the goals of this study, we considered AC, shyness, and NA. Regarding psychometrical properties, levels of reliability similar to reference studies (see Ellis & Rothbart, 2001; Muris & Meesters, 2009) were observed for the factors of interest across assessment points (Cronbach′s alpha from .50 to .71), and levels of correlation between points were ranged from Pearson′s $r = .45$ to $r = .74$ (all $p < .05$) for all the scales within our sample.

The Sensitivity to Punishment and Sensitivity to Reward Questionnaire− Junior version (SPSRQ−J; Torrubia, Garcia−Carrillo, Avila, Caseras, & Grande, 2008) is an instrument consisting of 30 dichotomous (yes/no) items which allows the two constructs related to Gray′s original theory (Gray, 1982) to be measured: sensitivity to reward and SP. For the purposes of this study we only used the SP scale, which showed satisfactory levels of reliability across assessment points in our sample (Cronbach′s alpha from .76 to .82). Correlations between the SP across measurement points were ranged from $r = .50$ to $r = .76$ (all $p < .05$) within our sample.

Procedure

The University Bioethics Committee approved all the study procedures. Once the research project was presented to school boards and participants provided the written consent forms signed by themselves and their legal guardians, they were gathered in a classroom. Two 1−hour sessions were carried out in which participants filled out the questionnaires, which were presented to participants in a counter−balanced order (in the same classroom, a random set of participants filled out the EATQ−R one day and the other group completed the SPSRQ−J and the RCADS; the questionnaires that each group did not fill out were completed on the following day). Measurement points were scheduled every six months over an 18−month period (starting when adolescents were in the third trimester of the first year of secondary school) and spanned three academic years of secondary schooling.

As a result, a total of 624 participants completed at least one questionnaire at measurement point T1, 731 participants at T2, 727 participants at T3,

and 623 participants at T4. Because our statistical approach allowed for the handling of temporal data series with intermittent missing data (Dantan, Proust−Lima, Letenneur, & Jacqmin−Gadda, 2008; Proust−Lima, Philipps, & Liquet, 2016), our final sample included 884 participants (13.01 years old on average, $SD = 0.56$; 51.80% of participants from high schools in rural areas). In terms of gender, 44.40% of participants were boys ($M = 13.07$ years old, $SD = 0.61$; 45% from schools in rural areas), and the rest were girls ($M = 12.94$ years old, $SD = 0.50$; 50.39% from schools in rural areas). No significant differences were found in terms of non−responders and responders for any sociodemographic, or temperamental− or anxiety−related variables.

Data analysis

A correlational analysis was performed to ensure that the FT factors considered in this study did not overlap with one another. To test the study hypotheses, we used the latent class mixed modeling (LCMM) approach based on a maximum likelihood framework with a modified Marquardt iterative algorithm and a Newton−Raphson like algorithm (Proust−Lima & Jacqmin−Gadda, 2005; Proust−Lima et al., 2016). This approach allows latent continuous and person−specific processes derived from multiple measurements over time to be handled. Moreover, LCMM provides a flexible way to deal with multivariate asymmetric distributions and latent covariates that fluctuate over time.

All of our hypotheses were tested in accordance with the structural equation modeling tradition i.e., comparing model fit. Thus, to deal with first aim of the study, we tested growth models derived from our criteria (RCADS scales) by comparing their fit to either linear or quadratic trajectories over time. The linear trajectory was based on standard linear mixed models; and the quadratic trajectory was modeled on two different standard link functions (see Proust−Lima et al., 2016), namely the rescaled beta CDF and the quadratic I−splines function with knots placed at criterion quantiles. Both functions have a quadratic shape, but the I−splines function requires more parameters be estimated, and therefore is more complex. Three indexes per subject were used to determine the goodness of fit (Proust−Lima, Sene, Taylor, & Jacqmin−Gadda, 2014): the sample−adjusted Bayesian information criterion (SABIC); the Akaike information criterion (AIC); and the mean universal approximate cross−validation criterion (UACV). Smaller model SABIC, AIC, and UACV values signify a better fit. We opted to use the UACV instead of other cross−validation estimates (e.g., cross−validation entropy) due to its flexibility when dealing with curvilinear outcomes and covariates with non−Gaussian distributions (Commenges, Proust−Lima, Samieri, & Liquet, 2015).

To address the second aim of our study, we tested for different anxious symptomatology courses (classes) within general trajectories. Thus, we tested latent process models with an increasing number of classes until finding either two consecutive unstable solutions (models without convergence) for each criterion or higher fit index values. The best fit was provided when we observed (Proust−Lima et al., 2014) the smallest SABIC, AIC, and UACV; means of posterior probabilities in each class greater than .70; and each class having a meaningful percentage of participants. Typically, 5% of a sample is used as a cut−off. However, because our study aimed to detect at−risk classes and prevalence rates for specific subclinical ADs with significant

functional impairment, we considered samples to be meaningful if they were made up of at least 2.5% of the study sample (see Roberts, Fisher, Turner, & Tang, 2015).

Finally, to satisfy the third aim of our study, we tested for different anxious symptomatology courses (classes) within general trajectories under the influence of covariates (i.e., gender and temperamental factors). To do this, we tested latent process models with an increasing number of classes until finding either two consecutive unstable solutions (models without convergence) for each criterion or higher goodness of fit index values. Models with covariates would add to the unconditional so-

lutions the effect of gender (under fixed effects) and the course of temperamental factors (under random effects). Similar rules used for considering the second aim of our study were followed to test whether the unconditional solution or the solution with covariates showed a better fit.

To run the analyses, R x64 3.0.1 was used (lcmm package; Proust−Lima et al., 2016).

Results

Descriptive Statistics

Mean scores and standard deviations of anxious symptomatology and temperamental factors are displayed in Table 1. Low to moderate correlations between temperamental factors were found at T1 (from Pearson′s $r = .05$, $p > .05$, for NA and shyness; to $r = .39$, $p < .01$, for SP and shyness), which were slightly higher at T2 (from $r = .09$, $p < .05$, for NA and shyness; to $r = .53$, $p < .01$, for SP and shyness) and T3 (from $r = -.04$, $p > .05$, for NA and shyness, to $r = .55$, $p < .01$, for SP and shyness); and they were low at T4 (from $r = .13$, $p > .05$, for NA and SP, to $r = .14$, $p < .01$, for NA and shyness). Regarding the association between the AC and FT factors, correlations (all significant at $p < .05$) ranged from $r = -.44$ to $r = -.15$ at T1, $r = -.42$ to $r = -.16$ at T2, $r = -.45$ to $r = -.21$ at T3, and $r = -.44$ to $r = -.16$ at T4 (the highest correlation with NA and the lowest with shyness for all assessment points).

The developmental course of anxiety and its trajectories Model comparison revealed that the latent process (trajectory) of overall anxious symptomatology better fit a quadratic solution, defined by the standardized beta CDF-modeled function, as it provided the lowest AIC, SABIC, and UACV.

Likewise, quadratic trajectories based on the beta function provided a better fit for social phobia, obsessive compulsive symptoms, and panic anxiety. A quadratic solution based on the I-splines function showed a better fit for separation anxiety and generalized anxiety (see Table 2). The results exhibited a curvilinear (quadratic) decreasing trajectory across the assessment points.

Regarding the second aim of the study, we found that the growth models identifying more than one time course for every criterion fitted better (see Table 3). Thus, it was found that the model with six courses of development fit best for total anxiety (mean of posterior probabilities in each class between .75 and .88). For social phobia symptomatology, the four−class model fit best (mean of posterior probabilities in each class between .78 and .87). Regarding the separation anxiety symptoms, the four−class model also provided the best fit (mean of posterior probabilities between .75 and .86). When considering the generalized anxiety symptomatology, the model with five classes fit best (mean of posterior probabilities between .74 and .82). For obsessive compulsive symptomatology, the model with three classes fit best (mean of posterior probabilities between .82 to .88). And finally, the four−class model was the one that fit best for the panic anxiety symptomatology (mean of posterior probabilities in each class between .72 to .85). See Figure 1 for more information on the classes that were identified. Also, estimates for the latent solutions with best fits are displayed in Table S1 (see the Supplementary material).

The developmental course of anxiety and the influence of risk factors In testing for how gender and temperamental factors allow for differentiating latent trajectory classes on AD symptomatology, model comparison revealed that the 2−class solution provided a better fit for total anxiety, social anxiety, and panic anxiety; and the 1−class model provided a better fit for the rest of each AD-specific symptomatology (see Table 4). This means that gender and temperamental factors play a significant role in the trajectory of all the anxiety−related criteria studied during our assessment period. The fact that models were constraint by gender and temperamental factors led to growth solutions with fewer classes showed a better fit than the nested ones. Moreover, these factors were also involved in determining the course of at−risk individuals in the case of total anxiety, social phobia, and panic.

Thus, and regarding the trajectory of the total anxiety symptomatology, the 2−class model

showed a good fit with mean of posterior probabilities in each class between .83 and .97, unlike the better fit found for the 6−class model when unconstraint solutions (models without covariates) were examined. The two classes that were identified were, firstly, a class consisting of 97.31% of the sample (the so−called decreasing−anxious class) with the intercept, NA, SP, the latent course of shyness, and the time*shyness interaction (with negative loading) having a significant influence on its trajectory; and secondly, a class made up of 2.69% of the sample (the at risk-anxious class) with the latent course of AC and NA (with a negative loading), and the time*NA interaction, as significant covariates (see Table S2 in the supplementary material to find the factorial loadings and related Wald statistics). Self−report trajectories of these groups are displayed in Figure 2, and the trajectories of the temperamental factors are shown in a better fit for the unconstraint models. Thus, for social phobia, the AIC, BIC, and UACV indexes were the lowest, and the mean of posterior probabilities in each class ranged from .79 to .93. The two classes that were identified were (see Figure 2) one consisting of 94.15% of participants (the so−called decreasing-social phobia class), with the intercept and the trajectories of SP, shyness, and NA as significant predictors, and another made up 5.85% of the sample (the so−called at risk−social phobia class), with the trajectories of AC, NA (with a negative loading), SP (with a negative loading), and shyness; gender; the interaction NA*measurement point, and the SP*measurement point, as predictors (see Figure 3 and Table S2 in the supplementary material). To mention a related piece of evidence, 56.25% of participants in the heightened-social phobia class were girls.

	Measurement point			
	T1	T2	T3	T4
Anxiety				
Global anxiety	32.61(16.47)	29.76(16.42)	27.85(16.09)	26.48(16.37)
Social phobia	11.63(5.85)	10.27(5.65)	9.98(5.65)	9.56(5.92)
Separation anxiety	2.53(2.79)	2.33(2.89)	2.03(2.80)	1.87(2.75)
GAD	7.67(3.71)	7.07(3.63)	6.44(3.54)	6.27(3.66)
OCD	5.17(3.50)	4.38(3.37)	3.90(3.35)	3.71(3.20)
Panic anxiety	6.06(5.03)	5.75(4.93)	5.51(4.83)	5.06(4.69)
Fearful temperament				
NA	3.07 (0.53)	3.00(0.57)	3.02(0.57)	3.00(0.57)
Shyness	3.62(0.73)	3.50(0.76)	3.56(0.75)	3.59(0.74)
SP	6.95(3.53)	6.32(3.57)	6.13(3.79)	5.67(3.75)
Effortful control				
AC	4.44(0.62)	4.45(0.61)	4.46(0.61)	4.44(0.60)

Table 1: Mean and standard deviations (between brackets) are displayed for each scale. Measurement points were scheduled each six months. GAD = Generalized anxiety disorder symptomatology; OCD = Obsessive compulsive disorder symptomatology; NA = Negative affectivity; SP = Sensitivity to punishment; AC = Attentional control.

In relation to the AD-specific trajectories, the 2−class models showed a better fit compared to the other nested models for social phobia and panic trajectories, unlike the 4-class solutions which showed

Finally, the 2−class solution was chosen to explain the latent process of panic symptomatology (see Table 4). The two classes identified, with means of posterior probabilities in each class ranging from .71 to .82 and both of which displayed a decreasing trajectory, were a class composed of most of the sample (85.26% of participants) with a lower symptomatology starting point (the so-called

low−panic class), and another class that included 14.74% of the sample and which showed a higher starting point for panic symptomatology (the so-called at risk−panic class). For the low−panic class, the significant covariates observed were the intercept, the trajectory of SP, and the trajectory of NA; on the other hand, for the at risk-panic class, gender was the only predictor to explain its trajectory (see Figure 3). In this group, 63.63% of participants were girls. For separation anxiety, obsessive-compulsive symptomatology, and generalized anxiety, the 1−class model fit better than the other nested models.

For separation anxiety, the trajectory of SP and shyness were revealed as significant covariates; for generalized anxiety, the significant covariates were the latent trajectories of SP, NA, and shyness and the shyness*measurement point interaction.

For obsessive compulsive symptomatology, it was found that the trajectories of SP and NA, and the NA*measurement point interaction were significant covariates to explain its trajectory (see Table S2 in the supplementary material to find the factorial loadings and related Wald statistics).

	LL	AIC	SABIC	UACV
Total anxiety				
Linear	-10979.10	21964.21	21969.02	12.41
Beta CDF	**-10797.79**	**21605.57**	**121613.61**	**12.24**
Splines	-10809.78	21635.56	21648.41	12.25
Social phobia				
Linear	-8430.09	16866.18	16871.01	9.47
Beta CDF	**-8290.91**	**16591.83**	**16599.86**	**9.38**
Splines	-8297.68	16611.37	16624.23	9.38
Separation anxiety				
Linear	-6541.85	13089.69	13094.53	6.35
Beta CDF	No convergence			
Splines	**-5524.60**	**11065.20**	**11078.07**	**6.10**
GAD				
Linear	-7214.29	14434.58	14439.41	8.04
Beta CDF	**-8290.91**	**16591.83**	**16599.86**	**9.38**
Splines	-8297.68	16611.37	16624.23	9.38
OCD				
Linear	-7006.58	14019.15	14023.99	7.58
Beta CDF	**-6597.48**	**13204.96**	**13213.01**	**7.50**
Splines	-6682.30	13380.61	13393.47	7.50
Panic anxiety				
Linear	-7992.76	15991.52	15996.35	8.68
Beta CDF	**-7346.58**	**14703.16**	**14711.20**	**8.36**
Splines	-744.34	14896.67	1517.55	8.36

Table 2: Model fit for every criterion was modelled by different link functions. Linear refers to standard linear mixed model function. Beta CDF and splines functions correspond to quadratic functions. Models in bold face showed the best fit. LL = Maximum log-likelihood estimator for the model convergence; AIC = Akaike information criterion; SABIC = sample-adjusted Bayesian information criterion; UACV = Mean universal approximate cross-validation estimator per subject. GAD = Generalized anxiety disorder symptomatology; OCD = Obsessive compulsive disorder symptomatology.

	LL	AIC	SABIC (adjusted Bayesian)	UACV
Total anxiety				
Class = 1	-10797.79	21605.57	21613.61	12.24
Class = 2	-10436.76	20889.53	20902.37	11.84
Class = 3	-10279.70	20581.39	20599.07	11.66
Class = 4	-10241.77	20511.55	20534.03	11.63
Class = 5	-10220.48	20474.97	20502.29	11.61
Class = 6	**-10198.19**	**20436.39**	**20468.51**	**11.60**
Class = 7	-10185.72	20404.22	20454.39	11.59
Social phobia				
Class = 1	-8290.91	16591.83	16599.86	9.38
Class = 2	-5202.37	10426.73	10404.74	5.98
Class = 3	-5153.71	10335.41	10307.42	5.76
Class = 4	**-5126.48**	**10286.96**	**10252.96**	**7.75**
Class = 5	-5153.71	10347.41	10307.42	5.76
Separation anxiety				
Class = 1	-5524.60	11065.20	11078.07	6.10
Class = 2	-7958.47	15932.95	15945.81	9.01
Class = 3	-7813.26	15648.52	15666.21	8.85
Class = 4	**-7777.07**	**15582.14**	**15604.66**	**8.81**
Class = 5	-7755.49	15544.98	15572.33	8.79
GAD				
Class = 1	-7070.13	14156.26	14169.14	7.98
Class = 2	-6780.50	13583	13561	7.66
Class = 3	-6695.02	13418.03	13390.04	7.56
Class = 4	-6672.13	13378.26	13344.26	7.54
Class = 5	**-6655.83**	**13351.66**	**13311.66**	**7.53**
Class = 6	-6644.72	13335.45	13289.44	7.52
OCD				
Class = 1	-6597.48	13204.96	13213.01	7.50
Class = 2	-6336.80	12689.60	12702.47	7.22
Class = 3	**-6255.29**	**12532.59**	**12550.27**	**7.13**
Class = 4	-6222.16	12472.32	12494.84	7.10
Panic anxiety				
Class = 1	-7346.58	14703.16	14711.20	8.36
Class = 2	-7021.69	14059.39	14072.25	7.99
Class = 3	-6887.47	13796.95	13814.63	7.84
Class = 4	**-6869.94**	**13767.88**	**13790.40**	**7.82**
Class = 5	-6860.66	13755.32	13782.67	7.82

Table 3: Models in bold face showed the best fit for each anxiety disorder symptomatology. LL = Maximum log-likelihood estimator for model convergence; AIC=Akaike information criterion; SABIC = Sample-information criterion; UACV = Mean universal approximate cross-validation estimator per subject. GAD = Generalized anxiety disorder symptomatology; OCD = Obsessive compulsive disorder symptomatology

Discussion

This study aimed to depict how anxious symptomatology evolved from early to middle adolescence, a concrete (sensitive) period in adolescence, considering a relatively short time scale (i.e., assessments every six months). Moreover, we intended to examine the varying developmental courses of anxiety during this period with particular interest in identifying the trajectories of individuals at-risk for ADs. Finally, we aimed to study the moderating effects of some risk factors on anxiety trajectory: gender and temperamental risk factors.

	LL	AIC	SABIC	UACV
Total anxiety				
Class = 1	-8348.62	16757.23	16803.18	10.24
Class = 2	**-8316.09**	**16718.17**	**16784.02**	**10.23**
Class = 3	No convergence			
Class = 4	No convergence			
Social phobia				
Class = 1	-6266.00	12588.00	12638.05	7.61
Class = 2	**-6201.08**	**12489.29**	**12554.16**	**7.60**
Class = 3	-6190.39	12492.79	12578.73	7.62
Class = 4	No convergence			
Class = 5	No convergence			
Separation anxiety				
Class = 1	**-4431.97**	**8729.94**	**8980.55**	**5.19**
Class = 2	No convergence			
Class = 3	No convercence			
GAD				
Class = 1	**-5517.16**	**11101.51**	**11151.01**	**6.73**
Class = 2	No convergence			
Class = 3	No convergence			
OCD				
Class = 1	**-5113.71**	**10287.41**	**10333.47**	**6.29**
Class = 2	No convergence			
Class = 3	No convergence			
Panic anxiety				
Class = 1	-5672.18	11404.35	11450.41	6.99
Class = 2	**-5661.34**	**11408.68**	**11474.68**	**6.98**
Class = 3	-5646.77	11405.53	11491.49	6.97
Class = 4	No convergence			
Class = 5	No convergence			

Table 4: For all the criterion, the latent class models without covariates are displayed. All solutions were modelled in base of quadratic link functions.Models in bold face showed the best fit for each anxiety disorder symptomatology. LL = Maximum log-likelihood estimator for model convergence; AIC = Akaike information criterion; SABIC = Sample-adjusted Bayesian information criterion; UACV = Mean universal approximate cross-validation estimator per subject. GAD = Generalized anxiety disorder symptomatology; OCD = Obsessive compulsive disorder symptomatology.

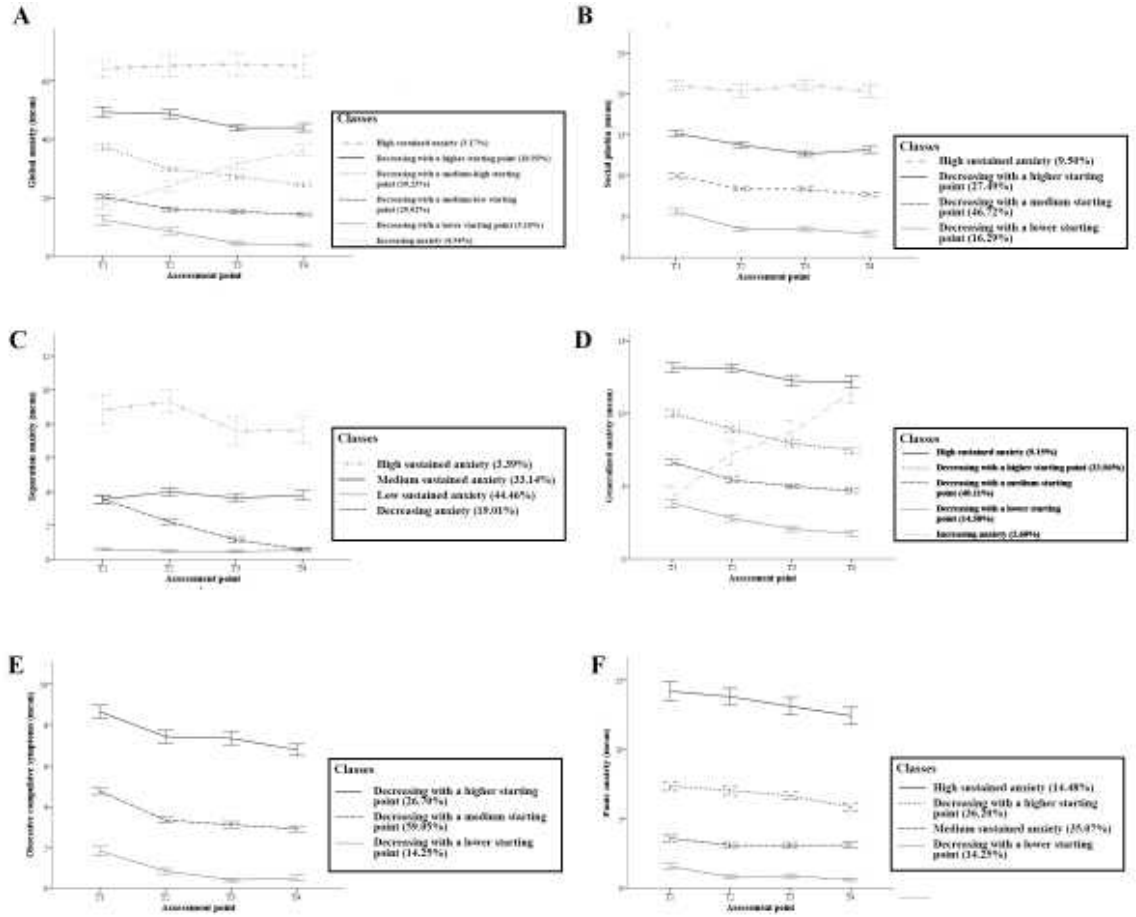

Figure 1: Figure A depicts the trajectory of the overall anxiety classes. Figure B depicts the trajectory of the social phobia classes. Figure C displays the scores of the separation anxiety classes. Figure D depicts the trajectory of the generalized anxiety classes. Figure E depicts the trajectory of the obsessive compulsive anxiety classes. Figure F displays the trajectory of the panic anxiety classes. Measurement points are displayed in the x-axis. They were taken every 6 months. Percentage of participants belonging to each class is displayed between brackets in legend boxes. Error bars depict the mean standard error.

The developmental trajectories of anxiety symptomatology

With regard to the first aim of the study, a curvilinear descending trajectory of anxiety symptomatology across our study period was found. This is in line with other studies (McLaughlin & King, 2015; Van Oort et al., 2009). Moreover, the shorter interval between assessments (just six months between consecutive assessments instead of one year or more) allowed for the curvilinear nature of the developmental course over our assessment period to be visualized: a course with a starting point at the highest level, going down with a relatively sharp slope but leveling off around the third assessment point. Thus, all the criterion solutions fit a curvilinear function better than the linear one (a curvilinear trajectory based on the beta CDF function fitted better for the majority of AD symptoms but a curvilinear trajectory based on the I−splines function for generalized anxiety and separation anxiety).

On the other hand, concerning the second aim of the study, we found varying trajectories in the course of anxiety symptoms across our assessment period. From a theoretical point of view, many factors (biological, behavioral, social, cognitive, etc.)

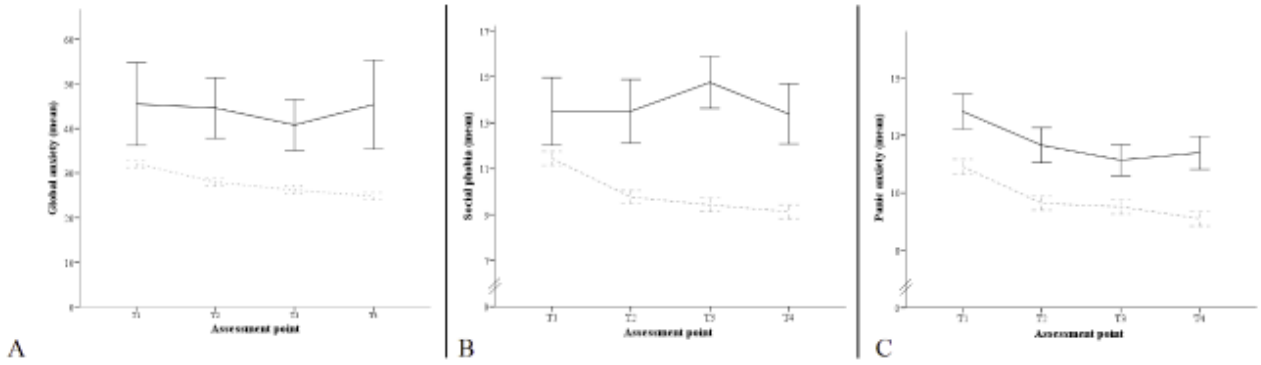

Figure 2: Figure in the A box depicts the trajectory of the total-anxiety classes. Figure in the B box depicts the trajectory of the social phobia classes. Figure in the C box displays the scores of the panic anxiety classes. These trajectories were based on the time passage plus the influence of temperamental factors and gender. Measurement points are displayed in the x-axis. They were taken every 6 months. Error bars depict the mean standard error. Dark solid line = trajectory of the heightened-anxious (box A and B) and high-anxious (box C) groups. Grey dashed line = trajectory of the decreasing-anxious (box A and B) or low-anxious (box C) groups.

may influence how anxiety symptomatology evolve in early and middle adolescence, thus leading to different trajectories of anxiety (Beesdo−Baum & Knappe, 2012; Weems, 2008). Most classes of anxiety courses showed a decreasing trajectory over time, but we also observed some that exhibited an increasing trajectory (for instance, overall anxiety and generalized anxiety). Moreover, some classes remained relatively stable over time. The classes that we observed related to separation anxiety and generalized anxiety were more varying and complex (e.g., the high-sustained anxiety class for separation anxiety and the increasing anxiety class for generalized anxiety); this could explain why the general course of both outcomes are better predicted by a more complex curvilinear-like function, such as the I−splines function. It is important to mention that for every criterion at least one class of trajectory surpassed the level of clinical severity across the assessment points (see Chorpita, Moffitt, & Gray, 2005), covering 3.39% of adolescents in the case of separation anxiety and up to 26.70% of the sample in the case of obsessive compulsive symptomatology.

Particularly interesting is the course of social phobia. Firstly, the general trajectory of social phobia across our assessment period showed anxiety levels exceeding the cutoffs for clinical meaningfulness in the first and second assessments (according to Chorpita et al., 2005, the cutoff point for clinical meaningfulness was a score of 10). Secondly, and regarding the varying developmental courses of social phobia that were found, we observed that individuals from two social phobia classes (more than 35% of individuals) exhibited clinical levels of social phobia symptomology at all assessment points. In turn, more than 83% of participants showed clinical levels of social phobia at the first assessment points. Altogether, these findings support the idea that the period from to middle adolescence is highly sensitive for the development of either a subthreshold or a full-blown social anxiety disorder (Beesdo-Baum & Knappe, 2012; Casey, Oliveri, & Insel, 2014; Kessler et al., 2012; Weems, 2008).

Moderating effects of gender and temperament on the time course of anxiety As proved by our findings, temperament had an influential role on the course of anxiety during the studied period. For all of the courses related to anxiety, we found a significant growth model with gender and/or temperamental−risk−factor covariates to explain at least the general course across assessments.

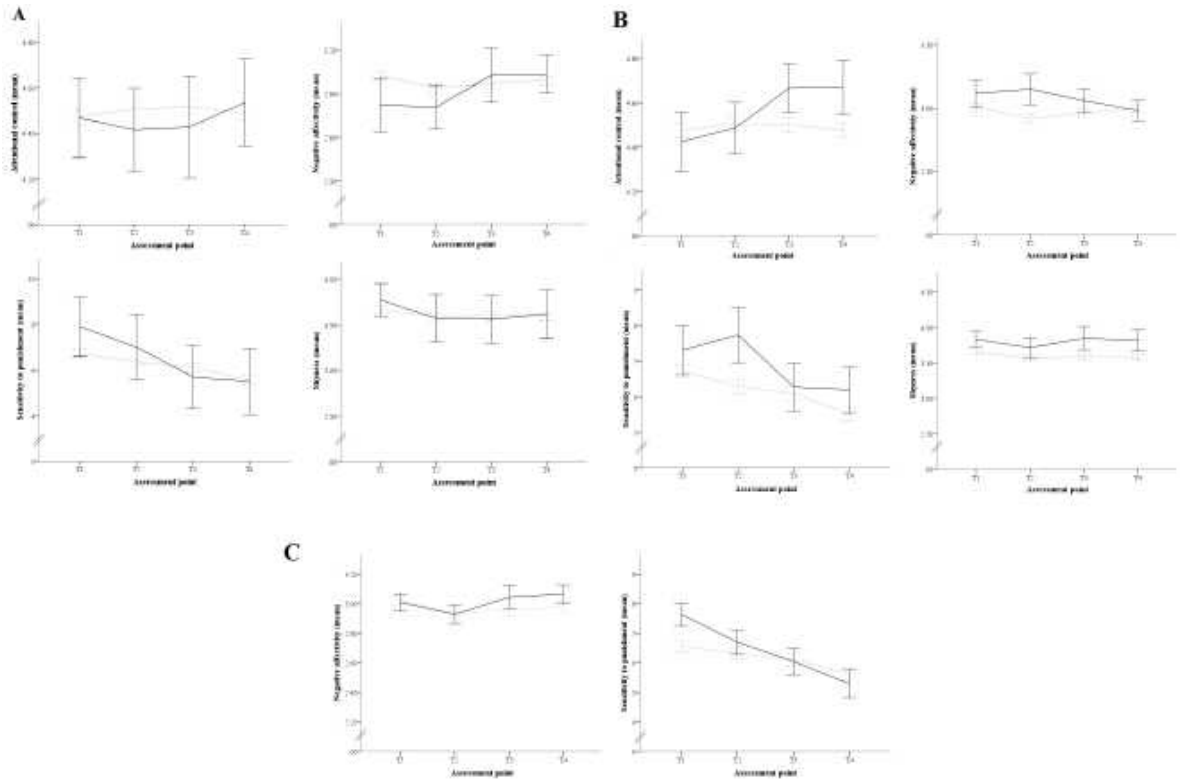

Figure 3: This set of figures depicts the trajectory of the temperamental risk factors that showed a significant explanatory role according to the class groups. Figures at the A box depict the trajectory of temperamental factors for the total anxiety groups. Figures at the B box depict the trajectory of the social anxiety groups. Figures at the C box depict the trajectory of temperamental factors for the panic anxiety groups. The trajectories of temperamental factors are depicted from left to right side as follow: attentional control, negative affectivity, sensitivity to punishment, shyness. Measurement points are displayed in the x-axis. They were taken every 6 months. Error bars depict the standard error of the mean. Dark solid line = trajectory of the heightened-anxious groups (or high-panic anxiety group). Grey dashed line = trajectory of the decreasing-anxious groups (or low-panic anxiety group).

As expected, more general temperament traits (NA and SP) were observed as significant predictors for overall anxiety and all of the AD-specific symptomatology time courses. Inhibited children and adolescents (those with high levels of FT) often exhibit high levels of AD symptoms throughout childhood and adolescence due to a higher propensity to react to varying stimuli with elevated arousal and avoidance (Bijttebier et al., 2009; Beauchaine & Thayer, 2015; Sportel et al., 2011). The modulating effect of the shyness trajectory has also been highlighted as crucial for the time course of overall anxiety symptomatology and some AD-specific symptom trajectories, too. This is probably due to the relevance of social concerns in early and middle adolescence (de Matos et al., 2016; Karevold et al., 2012). Finally, the role of gender and AC deserves a brief comment as they both have an influence on at−risk classes and not on classes with a normative course.

More specifically, it has been suggested that a risk profile consisting of high levels of FT and low levels of AC could be involved in the development of an AD (Bijttebier et al., 2009; De Pauw & Mervielde, 2010; Rothbart, 2007). And, this profile may be more pronounced in girls. The bulk of the evidence supporting this idea comes from transversal studies (Bornas, Balle, de la Torre-Luque, Fiol-Veny, & Llabres, 2015; Caouette & Guyer, 2014; Sportel et al., 2011).

Our study aimed at providing some evidence in this regard from a longitudinal point of view. Thus, and by means of growth mixture modeling, we firstly identified at-risk courses for three of our criteria: overall anxiety, social phobia, and panic anxiety. We found that only 2.69% of adolescents showed a heightened time course of overall anxiety. This low percentage may be because overall, unspecific anxiety symptomatology tends to be expressed as concrete disorders as adolescence progresses (see Weems, 2008). A moderating effect of AC and NA was observed to explain this at−risk trajectory (see the Figure 3). The time course of AC showed low levels at the first two assessment points and reached normative levels after. This result led to the trajectory of AC being a negative predictor for the trajectory of anxiety. We suggest that lower early levels of AC (even with subsequent normalization) depict a developmental at-risk course. Conversely, NA showed low levels early on but increased afterwards. In other words, adaptive low levels of NA at early stages do not preclude being at risk for ADs later in adolescence. These findings may point to an interplay of the two risk factors in depicting the heightened trajectory of anxiety symptomatology over time.

An at−risk group for social phobia, made up of 5.85% of the sample and with clinical scores of social phobia at all the assessment points, was found. All the FT−related factors studied, AC, and gender (basically, being a girl) were revealed to be significant predictors for explaining this group′s high−anxiety trajectory. Thus, low levels of AC and high levels of NA and SP at early assessment points explained the at−risk trajectory (see Figure 3, box B). Moreover, shyness showed a higher sustained trajectory among the at−risk adolescents throughout the assessment period. In this regard, general assumptions about higher FT and lower AC are proved to be correct considering the dynamic course of temperament throughout maturation (see Bijtebbier et al., 2009; Miers, Blote, de Rooij, Bokhorst, & Westenberg, 2013; Sportel et al., 2011). Regarding the panic-anxiety trajectory, a class of at−risk participants, which included 14.74% of the sample, was identified. These participants only showed clinical levels of panic anxiety at the first assessment point (surpassing the cut-off of 12 points). Moreover, being a girl was the only factor found to explain the at-risk course for panic. The moderating effect of gender on panic anxiety has already been highlighted, as some research has discovered related biological mechanisms (Cannon et al., 2013;Konishi et al., 2014). On the other hand, we suggest that prior experiences (in childhood and early adolescence) in terms of associations between bodily sensations, panic reactions, and prior panic attacks would be more relevant for the development of panic-related disorders than temperament (Asselmann, Wittchen, Lieb, Hofler, & Beesdo-Baum, 2014; Knapp, Frala, Blumenthal, Badour, & Leen-Feldner, 2013). However, further research should be conducted to test this conjecture.

Conclusions and directions for future research

So, the developmental course of anxious symptomatology displayed a curvilinear, decreasing trajectory across the transition to middle adolescence, but AD-specific symptomatology must be considered separately. Varying trajectories were identified for all AD-specific symptomatology. The role of FT was significant in depicting the time course of anxiety. FT factors and AC, as well as gender, were extremely relevant for explaining the heightened levels of anxiety (social phobia and panic symptomatology) across assessment points.

As a main strength, this study provides a piece of evidence on how anxiety symptomatology evolves during a sensitive period for the development of ADs, i.e., the transition to middle adolescence. Moreover, monitoring adolescents every six months allows clinicians and researchers to uncover relevant emotional changes in anxiety symptomatology over time which could otherwise have gone unnoticed if larger intervals between assessments had been used.

As a consequence, a more accurate picture on how anxiety evolves was obtained. Also noteworthy was the use of a robust methodological approach

(based on growth mixture models) that provided strong support for our findings, because between−subject and person−centered confounders are handled. Moreover, the subtypes of AD−specific symptomatology trajectory, not just overall anxiety, were addressed due to their relevance for maturation during adolescence. Finally, temperamental factors were dynamically considered, i.e., by means of their time courses.

As a limitation, this study was based on self-report assessments. This means that just a narrow view of how anxiety evolved over time and its day-to-day influence were considered. Future studies should integrate other assessment sources (parent and teacher reports, etc.). Moreover, we did not analyze the influence of contextual factors on the trajectory of anxiety symptomatology.

While our interest was on the influence of intrinsic factors (gender and temperament), regular contextual events were influencing the maturing adolescents (e.g., the adjustment to school). For this reason, a focus on posttraumatic stress disorder symptomatology, perhaps the disorder most influenced by external intense events, was discarded. However, further research should be conducted in order to determine how contextual events may influence the time course of anxiety. Similarly, biological correlates of maturational processes (e.g., scores on a maturity scale) could have been added to provide information on the specific stage of adolescence that each participant was at. Age and academic achievement were our determinants in this regard. Nevertheless, further research should include more biological biomarkers of adolescent maturity. Finally, our study was focused on a very concrete period in adolescence (the period from early to middle adolescence). Future research should expand the time frame and cover the whole of adolescence in order to determine how anxiety evolves as adolescents grow older.

Adolescence is a crucial time for the development of the self, and it influences adult wellbeing and health. Suffering an AD may have a decisive impact with consequences that endure for years and even decades. Our study has shed some light on when individuals may show at-risk profiles for these disorders so that psychosocial interventions can be carried out to prevent the development of a full-blown syndrome.

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Conflict of interests

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

References

Asselmann, E., Wittchen, H.-U., Lieb, R., Hofler, M., & Beesdo-Baum, K. (2014). Associations of fearful spells and panic attacks with incident anxiety, depressive, and substance use disorders: A 10−year prospective-longitudinal community study of adolescents and young adults. Journal of Psychiatric Research, 55, 8−14. doi:10.1016/j.jpsychires.2014.04.001

Balazs, J., Miklosi, M., Kereszteny, A., Hoven, C. W., Carli, V., Wasserman, C., ... Wasserman, D. (2013). Adolescent subthreshold-depression and anxiety: Psychopathology, functional impairment and increased suicide risk. Journal of Child Psychology and Psychiatry and Allied Disciplines, 54, 670-677. doi:10.1111/jcpp.12016

Beauchaine, T. P., & Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. International Journal of Psychophysiology, 98, 338−350. doi:10.1016/j.ijpsycho.2015.08.004

Beesdo−Baum, K., & Knappe, S. (2012). Developmental epidemiology of anxiety disorders. Child and Adolescent Psychiatric Clinics of North America, 21, 457−478. doi:10.1016/j.chc.2012.05.001

Betts, K. S., Bakker, P., Alati, R., McIntosh, J. E., MacDonald, J. A., Letcher, P., & Olsson, C. A. (2016). The natural history of internalizing behaviours from adolescence to emerging adulthood: Findings from the Australian Temperament Project. Psychological Medicine, 46, 2815-2827. doi:10.1017/S0033291716001495

Bijttebier, P., Beck, I., Claes, L., & Vandereycken, W. (2009). Gray′s Reinforcement Sensitivity Theory as a framework for research on personality-psychopathology associations. Clinical Psychology Review, 29, 421-430. doi:10.1016/j.cpr.2009.04.002

Bornas, X., Balle, M., de la Torre-Luque, A., Fiol−Veny, A., & Llabres, J. (2015). Ecological assessment of heart rate complexity: Differences between high- and low-anxious adolescents. International Journal of Psychophysiology, 98, 112−118. doi:10.1016/j.ijpsycho.2015.07.007

Caes, L., Fisher, E., Clinch, J., Tobias, J. H., & Eccleston, C. (2016). The development of worry throughout childhood: Avon Longitudinal Study of Parents and Children data. British Journal of Health Psychology, 21, 389-406. doi:10.1111/bjhp.12174

Cannon, D. M., Klaver, J. M., Klug, S. A., Carlson, P. J., Luckenbaugh, D. A., Ichise, M., & Drevets, W. C. (2013). Gender-specific abnormalities in the serotonin transporter system in panic disorder. International Journal of Neuropsychopharmacology, 16, 733-743. doi:10.1017/S1461145712000776

Caouette, J. D., & Guyer, A. E. (2014). Gaining insight into adolescent vulnerability for social anxiety from developmental cognitive neuroscience. Developmental Cognitive Neuroscience, 8, 65-76. doi:10.1016/j.dcn.2013.10.003

Capaldi, D. M., & Rothbart, M. K. (1992). Development and validation of an early adolescent temperament measure. Journal of Early Adolescence, 12, 153-173.

Casey, B. J., Oliveri, E., & Insel, T. (2014). A neurodevelopmental perspective on the Research Domain Criteria (RDoC) framework. Biological Psychiatry, 76, 350-353. doi:10.1016/j.biopsych.2014.01.006

Chorpita, B. F., Moffitt, C., & Gray, J. (2005). Psychometric properties of the Revised Child Anxiety and Depression Scale in a clinical sample. Behaviour Research and Therapy, 43, 309-322.

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of DSM-IV anxiety and depression in children: A revised child anxiety and depression scale. Behaviour Research and Therapy, 38, 835-855.

Commenges, D., Proust-Lima, C., Samieri, C., & Liquet, B. (2015). A universal approximate cross-validation criterion for regular risk functions. International Journal of Biostatistics, 11, 51-67. doi:10.1515/ijb-2015-0004

Copeland, W. E., Angold, A., Shanahan, L., & Costello, E. J. (2014). Longitudinal patterns of anxiety from childhood to adulthood: the Great Smoky Mountains Study. Journal of the American Academy of Child and Adolescent Psychiatry, 53, 21-33. doi:10.1016/j.jaac.2013.09.017

Dantan, E., Proust-Lima, C., Letenneur, L., & Jacqmin-Gadda, H. (2008). Pattern mixture models and latent class models for the analysis of multivariate longitudinal data with informative dropouts. International Journal of Biostatistics, 4, 14. doi:10.2202/1557-4679.1088

De Matos, M. G., Camacho, I., Reis, M., Costa, D., Galvao, D., & Aventura Social Team. (2016). Worries, coping strategies and well-being in adolescence: Highlights from HBSC study in Portugal. Vulnerable Children and Youth Studies, 11, 274-280. doi:10.1080/17450128.2016.1220655

De Pauw, S. S. W., & Mervielde, I. (2010). Temperament, personality and developmental psychopathology: A review based on the conceptual dimensions underlying childhood traits. Child Psychiatry and Human Development, 41, 313-329.

Ellis, L. K., & Rothbart, M. K. (2001). Revision of the Early Adolescent Temperament Questionnaire. Poster presented at the Biennial Meeting of the Society for Research in Child Development, Minneapolis, MN.

Essau, C. A., Lewinsohn, P. M., Olaya, B., & Seeley, J. R. (2013). Anxiety disorders in adolescents and psychosocial outcomes at age 30. Journal of Affective Disorders, 163, 125-132. doi:10.1016/j.jad.2013.12.033 Gray, J. A. (1982). The neuropsychology of anxiety: An enquiry into the functions of the septohippocampal system. New York, NY: Clarendon Press/Oxford Univer-

sity Press.

Gray, J. A., & McNaughton, N. (2000). The neuropsychology of anxiety. Oxford: Oxford University Press.

Hollenstein, T., & Lougheed, J. P. (2013). Beyond storm and stress: Typicality, transactions, timing, and temperament to account for adolescent change. The American Psychologist, 68, 444-454. doi:10.1037/a0033586

Karevold, E., Ystrom, E., Coplan, R. J., Sanson, A. V., & Mathiesen, K. S. (2012). A prospective longitudinal study of shyness from infancy to adolescence: Stability, age-related changes, and prediction of socio-emotional functioning. Journal of Abnormal Child Psychology, 40, 1167-1177. doi:10.1007/s10802-012-9635-6

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H.-U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. International Journal of Methods in Psychiatric Research, 21, 169-184.

Knapp, A. A., Frala, J., Blumenthal, H., Badour, C. L., & Leen-Feldner, E. W. (2013). Anxiety sensitivity and childhood learning experiences: impacts on panic symptoms among adolescents. Cognitive Therapy and Research, 37, 1151-1159. doi:10.1007/s10608-013-9558-8

Konishi, Y., Tanii, H., Otowa, T., Sasaki, T., Tochigi, M., Umekage, T., ... Okada, M. (2014). Gene x gene x gender interaction of BDNF and COMT genotypes associated with panic disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 51, 119-125. doi:10.1016/j.pnpbp.2014.01.020

Laceulle, O. M., Nederhof, E., Karreman, A., Ormel, J., & van Aken, M. A. G. (2012). Stressful events and temperament change during early and middle adolescence: The TRAILS Study. European Journal of Personality, 26, 276-284. doi:10.1002/per.832

Laceulle, O. M., Ormel, J., Vollerbergh, W. A. M., van Aken, M. A. G., & Nederhof, E. (2014). A test of the vulnerability model: Temperament and temperament change as predictors of future mental disorders − the TRAILS study. Journal of Child Psychology and Psychiatry, 55, 227-236. doi:10.1111/jcpp.12141

Leadbeater, B., Thompson, K., & Gruppuso, V. (2012). Co-occurring trajectories of symptoms of anxiety, depression, and oppositional defiance from adolescence to young adulthood. Journal of Clinical Child and Adolescent Psychology, 41, 719-730. doi:10.1080/15374416.2012.694608

Letcher, P., Sanson, A., Smart, D., & Toumbourou, J. W. (2012). Precursors and correlates of anxiety trajectories from late childhood to late adolescence. Journal of Clinical Child & Adolescent Psychology, 41, 417-432. doi:10.1080/15374416.2012.680189

McLaughlin, K. A., & King, K. (2015). Developmental trajectories of anxiety and depression in early adolescence. Journal of Abnormal Child Psychology, 43, 311-323. doi:10.1007/s10802-014-9898-1

Miers, A. C., Blote, A. W., de Rooij, M., Bokhorst, C. L., & Westenberg, P. M. (2013). Trajectories of social anxiety during adolescence and relations with cognition, social competence, and temperament. Journal of Abnormal Child Psychology, 41, 97-110. doi:10.1007/s10802-012-9651-6

Muris, P., & Meesters, C. (2009). Reactive and regulative temperament in youths: Psychometric evaluation of the Early Adolescent Temperament Questionnaire-Revised. Journal of Psychopathology and Behavioral Assessment, 31, 7-19. doi:10.1007/s10862-008-9089-x

Nelemans, S. A., Hale III, W. W.,, Branje, S. J. T., Raaijmakers, Q. A. W., Frijns, T., van Lier, P. A. C., & Meeus, W. H. J. (2014). Heterogeneity in development of adolescent anxiety disorder symptoms in an 8-year longitudinal community study. Development and Psychopathology, 26, 181-202. doi:10.1017/S0954579413000503

Olino, T. M., Klein, D. N., Lewinsohn, P. M., Rohde, P., & Seeley, J. R. (2010). Latent trajectory classes of depressive and anxiety disorders from adolescence to adulthood: Descriptions of classes and associations with risk factors. Comprehensive Psychiatry, 51, 224-235.

doi:10.1016/j.comppsych.2009.07.002

Proust-Lima, C., & Jacqmin-Gadda, H.(2005). Estimation of linear mixed models with a mixture of distribution for the random-effects. Computer Methods and Programs in Biomedicine, 78, 165−173. doi:10.1016/j.cmpb.2004.12.004

Proust-Lima, C., Philipps, V., & Liquet, B. (2016). Estimation of extended mixed models using latent classes and latent processes: The R package lcmm. Ithaca, NY: Cornell University Library.

Proust−Lima, C., Sene, M., Taylor, J. M., & Jacqmin−Gadda, H. (2014). Joint latent class models for longitudinal and time−to−event data: A review. Statistical Methods in Medical Research, 23, 74−90. doi:10.1177/0962280212445839

Rapee, R. M., & Coplan, R. J. (2010). Conceptual relations between anxiety disorder and fearful temperament. In H. Gazelle & K. H. Rubin (Eds.), Social anxiety in childhood: Bridging developmental and clinical perspectives. New Directions for Child and Adolescent Development, 127, 17−31. San Francisco: Jossey−Bass.

Roberts, R. E., Fisher, P. W., Turner, J. B., & Tang, M. (2015). Estimating the burden of psychiatric disorders in adolescence: the impact of subthreshold disorders. Social Psychiatry and Psychiatric Epidemiology, 50, 397-406. doi:10.1007/s00127-014-0972-3

Rothbart, M. K. (2007). Temperament, development, and personality. Current Directions in Psychological Science, 16, 207-212.

Rubin, K. H., Coplan, R. J., Bowker, J. C., & Menzer, M. (2011). Social withdrawal and shyness. In P. K. Smith & C. Hart (Eds.), Blackwell′s handbook of childhood social development (2nd ed) (pp. 434−452). Guichester: John Wiley & Sons, Ltd.

Sportel, B. E., Nauta, M. H., de Hullu, E., de Jong, P., & Hartman, C. A. (2011). Behavioral inhibition and attentional control in adolescents: Robust relationships with anxiety and depression. Journal of Child and Family Studies, 20, 149−156.

Susa, G., Pitica, I., Benga, O., & Miclea, M. (2012). The self regulatory effect of attentional control in modulating the relationship between attentional biases toward threat and anxiety symptoms in children. Cognition and Emotion, 26, 1069-1083. doi:10.1080/02699931.2011.638910

Torrubia, R., Garcia-Carrillo, M., Avila, C., Caseras, X., & Grande, I. (2008). A junior questionnaire for the measurement of sensitivity to punishment and sensitivity to reward. Unpublished manuscript, Autonomous University of Barcelona.

Tortella-Feliu, M., Balle, M., & Sese, A. (2010). Relationships between negative affectivity, emotion regulation, anxiety, and depressive symptoms in adolescents as examined through structural equation modeling. Journal of Anxiety Disorders, 24, 686-693. doi:10.1016/j.janxdis.2010.04.012

Van Oort, F. V. A., Greaves-Lord, K., Ormel, J., Verhulst, F. C., & Huizink, A. C. (2011). Risk indicators of anxiety throughout adolescence: The TRAILS study. Depression and Anxiety, 28, 485-494. doi:10.1002/da.20818

Van Oort, F. V. A., Greaves-Lord, K., Verhulst, F. C., Ormel, J., & Huizink, A. C. (2009). The developmental course of anxiety symptoms during adolescence: The TRAILS study. Journal of Child Psychology and Psychiatry, 50, 1209-1217. doi:10.1111/j.1469-7610.2009.02092.x

Weems, C. F. (2008). Developmental trajectories of childhood anxiety: Identifying continuity and change in anxious emotion. Developmental Review, 28, 488-502. doi:10.1016/j.dr.2008.01.001

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ORIGINAL ARTICLE

Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment

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KEYWORDS
Anxiety;
Adolescence;
Allometric control;
Fractality;
Ex post facto study

Abstract *Background/Objective*: Anxiety symptoms in adolescence have been found to be associated with heart rate variability (HRV) linear features, but more basic properties of the cardiac system remain unexplored. This study focused on the fractal nature of 90 minute-long interbeat fluctuations from 24 adolescents with high anxiety and 26 with low anxiety to (a) evaluate if allometric scaling exponents and linear HRV measures allow for distinction between groups, and (b) assess the associations between these measures and sensitivity to punishment (SP), a temperamental characteristic strongly correlated with anxiety. *Method*: Cardiac functioning was recorded and allometric exponents and vagally mediated HRV as indexed by the high frequency (HF) band power were calculated. *Results*: While the exponents from the high anxiety group were significantly higher than those from low anxiety participants ($p < .05$), just marginal differences were found for the HF measure ($p = .057$). Furthermore, exponents were positively correlated with SP scores and several anxiety scale scores, but no more correlations were found. *Conclusions*: These results show that beyond parasympathetic functioning, basic properties of the cardiac system may be altered in young, anxious adolescents. These properties, therefore, can provide useful information for assessing adolescents at risk of anxiety disorders.

PALABRAS CLAVE
Ansiedad;
adolescencia;
control alométrico;
fractalidad;
estudio *ex post facto*

Fractalidad cardiaca en adolescentes tempranos: sus asociaciones con la sintomatología ansiosa y la sensibilidad al castigo

Resumen *Introducción/Objetivo*: Se ha asociado la existencia de sintomatología ansiosa con algunas propiedades lineales de la variabilidad cardiaca (VC), sin prestar demasiada atención a propiedades más esenciales del sistema cardiaco, como su naturaleza fractal. En este trabajo se

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pretendía evaluar si medidas de fractalidad (exponentes alométricos) y medidas de VC (potencia en la banda de altas frecuencias, AF) permitían distinguir entre 24 adolescentes con alta sintomatología ansiosa y 26 adolescentes con baja. Además, se perseguía explorar las asociaciones de estas medidas con sensibilidad al castigo (SC), un factor de riesgo para ansiedad. *Método*: Se tomó la actividad cardiaca de los adolescentes en contexto ecológico y se calcularon dichas medidas sobre registros de 90 minutos. *Resultados*: Se encontraron exponentes alométricos significativamente mayores para los adolescentes con alta ansiedad ($p < 0,05$), sin observarse diferencias significativas en potencia de AF ($p = 0,057$). Además, sólo se encontraron correlaciones positivas significativas entre los exponentes alométricos con SC, y dichos exponentes con varias escalas de ansiedad. *Conclusiones*: Estos resultados muestran que propiedades más básicas del sistema cardiaco parecen estar alteradas en adolescentes ansiosos más allá de la mera influencia parasimpática. Estas propiedades pueden aportar información relevante para la detección y prevención de trastornos de ansiedad.

Fluctuations in the length of successive interbeat intervals are not random but fractal-like, i.e. similar patterns of fluctuations can be seen at different temporal resolutions (Goldberger, 1996; West, 2010; West, Brown, & Enquist, 1999). In other words, there is multistability and the heart does not beat within one single time scale: different set points (at different time scales) are available to the heart system providing flexibility to respond effectively to the incoming demands. In addition, altered fractal dynamics in the heartbeat have been found to be associated with disease and aging (Goldberger et al., 2002; Sturmberg, Bennett, Picard, & Seely, 2015).

Based on these findings, West (2006) introduced the concept of allometric control. Classical explanations for heart beat fluctuations embraced homeostasis as the key control mechanism of the seemingly random deviations from the mean interbeat interval length. Homeostatic control would operate at just one single, real-time scale and the system producing those fluctuations would be memoryless. Allometric control, conversely, means that there are control mechanisms that operate at different time scales and that the system has a built-in long-term memory. West (2006) suggested that scale-free fluctuations would emerge mainly from the interaction of the two branches of the autonomous nervous system (ANS), the sympathetic and the parasympathetic branches (see also Ivanov, Chen, Hu, & Stanley, 2004; Lehrer & Eddie, 2013).

Fractal objects look the same at different spatial scales or resolutions. Similarly, fractal time series should look the same when seen at different temporal resolutions. Therefore, perhaps the most intuitive way to check if a signal shows multistability (and hence to get some evidence that it is under allometric control mechanisms) is to examine this signal at different time resolutions and test the invariance of the relationship between its mean and its variance along those resolutions. This procedure is very similar to the one introduced by B. Mandelbrot to demonstrate the fractal nature of the coast of Great Britain (Mandelbrot, 1967). The relationship between the length of the coast and the scale that we use to measure it remains invariant, so that when we plot the length obtained using each scale on log-log axes a straight line can be traced that crosses all these points, and the slope of this line (which is invariant all along the line) provides a calculation of the fractal dimension of the shape of the coast. Allometric aggregation (West, 2006) allows for testing fractality in temporal objects (i.e. time series) by plotting the means and the variances for successively aggregated values (i.e. different resolutions) from a time series on log-log axes. The slope of the straight line that fits these values on the plot is the scaling exponent that gives a quantitative measure of the scale invariance present in the data (a detailed explanation of the allometric aggregation procedure can be found in the Methods section). This procedure is more straightforward and less time-consuming than other methods like detrended fluctuation analysis (Peng, Havlin, Stanley, & Goldberger, 1995) and could be performed using any standard spreadsheet. This may seem trivial, but sophisticated mathematical procedures are not easy for most Clinical Psychology researchers, and this is probably one of the reasons that could explain why they are reluctant to use nonlinear measures based on those complex procedures. However the main reason why most research addressing the relationships between the heart system and anxiety has not taken into account the fractal-like properties of the cardiac system is the leading existence of the influential models developed by Thayer and Lane (2000, 2009) or Porges (2001).

These models emphasize the parasympathetic or vagally-mediated heart rate variability (vmHRV). In truth, there is a large body of research pointing at an association between diminished vmHRV and several anxiety disorders (see Beauchaine & Thayer, 2015; Chalmers, Quintana, Abbott, & Kemp, 2014). Furthermore, most studies on general population and clinical samples have found a positive association between the behavioral inhibition system (BIS) sensitivity or sensitivity to punishment (SP) and anxiety symptomatology (Bijttebier, Beck, Claes, & Vandereycken, 2009; Panayiotou, Karekla, & Panayiotou, 2014), and associations have also

been found between the BIS sensitivity and vmHRV (Balle, Tortella-Feliu, & Bornas, 2013; Beauchaine, 2001), although Kristensen, Oerbeck, Torgersen, Hansen, and Wyller (2014) could not find any evidence of autonomous alterations in anxious children, measuring from the 0.15–0.4 Hz frequency domain band (usually called the high frequency or HF band). This band exclusively, or overwhelmingly, reflects the vagal influence on HR (Shaffer, McCraty, & Zerr, 2014). It should be noticed that the HF band power is usually calculated using short recordings taken in lab settings. These measurements may not reflect the properties of the heartbeat in the ecology of everyday life.

In agreement with Nardelli et al. (2015) who showed the interest of using nonlinear analysis of HRV for the study of psychological dimensions, we think that there is room for the study of the relationships between complex heart properties other than vmHRV and anxiety symptoms, as well as for the study of the associations between those properties and BIS sensitivity. Recently, in a study focused on attentional orienting Balle, Morillas, Tortella-Feliu, and Bornas, (2015) pointed out that the influence of the parasympathetic system should be considered within the complex, multiscale structure of the heartbeat system.

This study was aimed at (a) elucidating if heartbeat allometric-related scaling properties are different in low anxiety adolescents when compared to high anxiety adolescents, and (b) comparing the associations between physiological indexes of the heartbeat (vmHRV and scaling exponents) and anxiety symptoms, as well as sensitivity to punishment. Heartbeat activity was recorded in everyday life conditions to ensure the ecological validity of the measures calculated on the resulting interbeat intervals time series.

Based on previous results (Bornas, Balle, de la Torre-Luque, Fiol-Veny, & Llabrés, 2015), and taking into account the relationship between fractal dimension (FD) and scaling exponents *h* (*FD* = 2-*h*), our hypothesis as to the first aim was that heartbeat scaling exponents from high anxiety adolescents would be higher than the exponents from their low anxiety counterparts. Regarding the associations between physiological and psychological measures, we hypothesized that scale-free behavior (or scaling) in long ecological recordings would be more strongly associated with anxiety and SP than vmHRV, as the former reflects a more basic property and more fundamental control mechanisms (i.e. allometric control) of the heartbeat than the latter.

Method

This study is defined by an *ex post facto* design with two groups, one of them a quasi-control group (Montero & León, 2007).

Participants

This study was part of a longitudinal project among young adolescents (Bornas, Llabrés, Balle, de la Torre-Luque, & Fiol-Veny, 2014). We initially recruited 192 participants from 13 high schools across the island of Mallorca (Spain). All of them were Caucasian, from middle socioeconomic backgrounds and both urban and rural areas. All of them consented to take part in this study as did their parents/legal guardians. In order to match the requirements to test our hypotheses, the final sample was selected by means of the Revised Child Anxiety and Depression Scale (RCADS; Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000). Therefore, the participants selected were the ones who showed scores above the 75th percentile (scores $\geq$ 30) in total anxiety symptomatology (they made up the so-called high anxiety group) and those with scores below the 25th percentile (scores $\leq$ 22) as well (they made up the so-called low anxiety group). Additionally, some exclusions were taken into account, which were diagnosis of severe mental retardation; diagnosis of neurological, developmental, or psychiatric disorder (American Psychiatric Association, 2000), or being diagnosed with any severe cardiovascular or respiratory disease. As a result of selection, and after applying all the study protocols, the sample was composed of 24 high anxiety participants (17 females, $M = 12.89$ years old, $SD = 0.42$; and 7 males, $M = 12.99$ years old, $SD = 0.40$) and 26 low anxiety participants (14 females, $M = 13.03$ years old, $SD = 0.60$; and 12 males, $M = 12.95$ years old, $SD = 0.55$). It was significantly different proportions according to gender in the high anxiety group, χ^2 (1) = 4.17, $p = .041$, $\phi = .42$; but not regarding the low anxiety group, χ^2 (1) = .15, $p > .69$, $\phi = .09$. None of the potential participants were ruled out due to the exclusion criteria (for further details, see Bornas et al., 2015).

The University Bioethics Committee approved all the procedures conducted in this research.

Instruments

- The Mini-International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid; Sheehan et al., 1998) was used to evaluate the presence of psychiatric disorders. The tool was administered to each participant by a trained postgraduate psychologist in face-to-face sessions. M.I.N.I. Kid consists of a structured diagnostic interview for children and adolescents from 6 to 17 years old to assess 25 psychiatric disorders in keeping with the DSM-IV and ICD-10 criteria: major depressive episode, suicide risk, dysthymia, (hypo) manic episode, panic disorder, agoraphobia, separation anxiety disorder, social phobia, specific phobia, obsessive-compulsive disorder, posttraumatic stress disorder, alcohol dependence/abuse, substance dependence/abuse (non-alcohol), tic disorders, attention-deficit hyperactivity disorder, conduct disorder, oppositional defiant disorder, psychotic disorders, anorexia nervosa, bulimia nervosa, generalized anxiety disorder, and adaptive disorders. The interview evaluates the bulk of these psychiatric disorders by two to four initial screening questions. When these questions are answered negatively the diagnosis is ruled out. Conversely, positive responses to them involved administering all the disorder-related questions in order to get further information about whether the diagnosis was present.
- The Revised Children's Anxiety and Depression Scale (RCADS; Chorpita et al., 2000; Sandín, Valiente, & Chorot, 2009) was used to assess anxiety symptoms. The questionnaire allows different anxiety disorder symptomatology (separation anxiety disorder, social anxiety disorder,

generalized anxiety disorder, panic disorder, obsessive compulsive disorder) and major depressive disorder to be explored, by means of 47 self-report items. Additionally, RCADS provides a composite scale of anxiety symptomatology. The RCADS asks for respondents to rate how often each symptom is present regularly. Symptoms are scored by means of a 4-point Likert-type scale (from 'never' to 'always'). The internal consistency of the overall anxiety scale within this study sample was $\alpha = .93$. For the purposes of this study, scales related to anxiety symptomatology were used.

- The Sensitivity to Punishment and Sensitivity to Reward Questionnaire Junior (SPSRQ-J; Torrubia, García-Carrillo, Ávila, Caseras, & Grande, 2008) was used to measure temperamental tendencies in keeping with the Grey's framework. The instrument is made up of 30 dichotomous items (yes–no scale of response). Two scales can be extracted using this instrument: sensitivity to punishment (SP), and sensitivity to reward (SR). In this study, only the SP scale (Cronbach's $\alpha = .81$) was relevant to the hypothesis and therefore used for analyses.
- Cardiac measures. In addition to the mean heart rate (HR) the standard deviation of the averages for normal-to-normal beat intervals in all 5-minute segments (SDANN) of the entire recording was provided as a measure of the time-domain HRV. Vagally-mediated HRV (vmHRV). The HF (0.15-0.40 Hz) power was calculated using Kubios HRV 2.1 Release software (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014), an advanced tool for analyzing the variability of heartbeat intervals. HF-related data were normalized by means of log-linear transformations (lnHF). The scaling exponents of each interbeat interval (IBIs) time series (see the Data acquisition devices and Data preprocessing section below) were calculated by means of the allometric aggregation method. Allometric aggregation examines the invariance of the relationship between the mean and the variance, or the standard deviation, of a data series as the data points are iteratively aggregated, thus decreasing the resolution (the scale) of the series. First, the mean and the standard deviation (the square root of the variance) of the time series of length N ($x_1, x_2, x_3, \ldots, x_n$) are calculated. Second, every two adjacent points are aggregated ($x_1 + x_2, x_3 + x_4, \ldots, x_{n-1} + x_n$) to get a time series of length $N/2$, and the mean and the standard deviation of this time series are calculated. This aggregation process is repeated for 3, 4, 5, and usually up to $N/10$ adjacent data points. As the IBI time series were rather long (around 9000 values) the aggregation process was repeated for 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 adjacent data points in this study. Figure 1 depicts the logarithm of the mean (horizontal axis) versus the logarithm of the standard deviation (vertical axis), for each level of aggregation of an IBI time series from one participant. At the extreme left, the first dot denotes the value of the mean and standard deviation obtained using all data. Moving from left to right, the next dot corresponds to the mean and standard deviation for the time series with ten adjacent values added together, and so on. As can be seen, when the number of aggregated values increases, the relationship between mean and standard deviation remains the same (invariant) and can therefore be described as a linear relationship between log (mean) and log (standard deviation). The slope of the line representing that linear relationship is the scaling exponent h. Notice that the scaling exponent for random fluctuations is 0.50, and for deterministically regular processes is 1 (in both cases the time series would not be self-similar or fractal). If we take the variance instead of the standard deviation then the slope of the line would be $2h$.

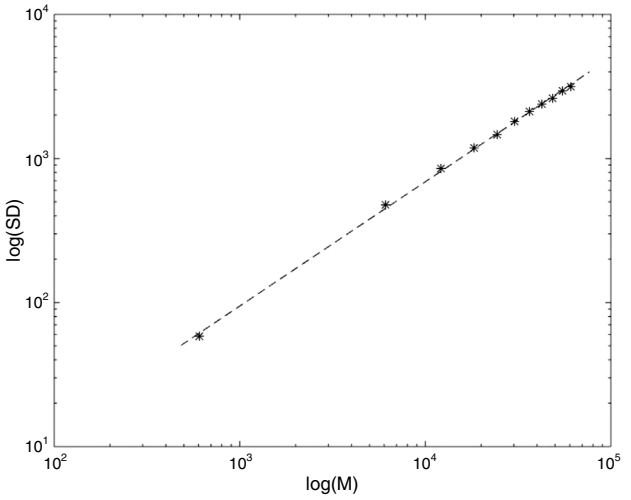

Figure 1 Logarithmic plot of mean (x axis) and standard deviation (y axis) for ten levels of aggregation (1, 10, 20 ... 100) of the interbeat intervals time series from one participant ($h = 0.86$).

Procedure

To conduct the study, we randomly selected 20 high schools across the Mallorca Island (Spain) and contacted with their boards to give information about the protocols, requirements, etc., and to encourage them to participate. Thirteen high schools accepted to participate.

Online versions of the aforementioned self-reports were administered to participants. Additionally, parents and high school boards provided some socio-demographic data. Afterwards, the M.I.N.I. Kid was applied individually to each participant. Once it was ratified that participants matched the study criteria, cardiac recordings were scheduled. These recordings were conducted on a regular class day within the academic context, but there were no programmed exams or physical or extraordinary activities scheduled at the time the device was recording. Therefore, participants left the classroom in the early morning (around 8:30 a.m.) and went to a secluded room. First of all, researchers ensured that participants had not consumed alcohol, drugs and/or caffeinated beverages in the 4 hours prior to their participation in the study and also that there was no acute illness or menstruation (recording was postponed to another day just in case of acute illness/menstruation). Afterwards, participants were weighed and measured, and body mass index (BMI) was subsequently calculated. Two electrodes and the portable heart-recording device (see description below) were put on the participant's chest later on and their correct functioning was checked. At this moment, the participants returned to their classroom to attend regular

class. Cardiovascular functioning was taken throughout two hours continuously and after this time (around 10.30 a.m.) the researcher brought the participant to the secluded room again and removed the device. Finally, recorded data were transferred and stored on a computer.

Data acquisition devices and data preprocessing

Cardiac recordings were taken by using the Firstbeat Bodyguard 2© (Firstbeat Technologies Ltd., Jyväskylä, Finland) device. This portable device was attached to 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 device continuously recorded beat-to-beat heart rate (i.e. interbeat intervals) at a sample rate of 1000 Hz. As mentioned above, recordings were 2 hours long and the resulting IBI time series were processed and analyzed offline. The first 15 minutes of each time series as well as the last 15 minutes were removed from the analyses in order to avoid data being influenced by the experimental protocols carried out in this study (adaptation to the device, leaving the classroom to go to the secluded room, and so on).

Hence, 90 minute-long time series were processed and analyzed (the number of IBIs in each time series depended on the participant's heart rate). Time series were filtered by using the Physionet (Goldberger et al., 2000) HRV toolkit (http://www.physionet.org/tutorials/hrv-toolkit/) as follows. First, any IBI less than 400 ms or greater than 1100 ms was ignored. Next, using a window of 11 intervals (5 intervals on either side of the central interval), the average over the window was calculated excluding the central interval. If the central interval lay outside 20% of the window average, this interval was excluded, then the window went forward to the next interval. As a result, the total excluded IBIs was 7,541 (out of 556,407, i.e. 1.35%). HR, SDANN, lnHF power and scaling exponents were calculated on these time series.

Analytic strategy

Firstly, the influence of socio-demographic variables on cardiac measures was tested by analyzing the presence of significant differences between study groups. Accordingly, we used the χ^2 independency tests for categorical variables, and between-group *t*-tests for BMI. If any of these variables showed different distributions between groups, that one would be controlled as covariate. Secondly, *t*-tests were used to compare the mean vmHRV (lnHF) and scaling exponents from the low and high anxiety groups. Effect size estimations were calculated by using Cohen's *d* statistic for numerical variables and Cramer's ϕ for categorical ones (for further details, see Fritz, Morris, & Richler, 2012).

Finally, a correlational analysis was performed by means of bivariate Pearson's product-moment correlations between cardiac measures and psychological measures (self-reported anxiety and sensitivity to punishment scores). Normality assumptions were previously checked to account for running those analyses.

All analyses were run using the IBM SPSS Statistics v. 20.0.0 statistical package.

Results

Table 1 summarizes the socio-demographic features of samples presented by the study groups. Moreover, scores arising from the psychological collected data are shown. As can be observed, there were no significant differences between groups, but expected significant differences in terms of psychological measures ($p < .05$ for all these measures).Regarding the cardiac measures, non-significant differences were found between groups with respect to the mean heart rate and SDANN throughout the recording (see Table 1). Conversely, significant differences were found between the scaling exponents *h* from the study groups ($p < .01$), observing higher exponents in the high anxiety group in comparison to low anxiety group. On the other hand, differences in lnHF power were marginally significant ($p = .057$) as the lnHF power of the low anxiety group was slightly higher than the lnHF power of the high anxiety group.All psychological variables and cardiac measures showed a normal-like distribution. Pearson's correlation analyses (see Table 2) revealed significant associations between the scaling exponents and the scores derived from the sensitivity to punishment and the overall anxiety scale, as well as the scales related to social anxiety disorder, generalized anxiety, and obsessive compulsive disorder ($p < .05$, for all correlations). No significant associations were found between the lnHF and the psychological variables studied.

Discussion

The association between anxiety symptoms and diminished vmHRV has received increasing attention over the last decades, following the influential theoretical models developed by Porges (2001) and Thayer and Lane (2000, 2009). Despite the valuable information provided by this research, with the growth of nonlinear dynamic systems-based research (Bornas, Noguera, Pincus, & Buela-Casal, 2014; Bravi, Longtin, & Seely, 2011; Nardelli et al., 2015; Stadnitski, 2012; West, 2010), the search for nonlinear biomarkers of psychological disorders has become a promising complementary area where complex cardiac features associated with anxiety symptoms might be found also. In this study we evaluated the scaling properties of the heartbeat output from low and high anxiety groups of adolescents, and the associations between anxiety symptomatology and SP, on one hand, and vmHRV and heartbeat scaling exponents on the other.

As expected, inter-group comparisons revealed significantly higher scaling exponents for the high anxiety group while vmHRV was only marginally reduced in this group when compared to the low anxiety group. On the other hand, we hypothesized that the associations between the scaling exponents and the psychological variables should be stronger than those between these variables and the vmHRV since the former reflect more fundamental properties (e.g. multistability) of the cardiac dynamics than the latter.

The results from our correlational analyses provide some evidence that support this hypothesis as no correlations were found for vmHRV whereas several anxiety symptoms (those specifically seen in either social anxiety disorder, generalized anxiety, or OCD symptoms), the total anxiety

Table 1 General socio-demographic, psychological and cardiac descriptive statistics for the study groups.

	Low anxiety adolescents	High anxiety adolescents	t/χ^2	*df*	*p*	Effect size [†]
Gender (% girls)	53.85	70.80	1.53	1	.23	.18
Age	12.96 (0.44)	12.87 (0.41)	.81	46	.42	−.21
Body mass index	20.04 (4.30)	19.87 (2.74)	.15	41.24	.88	−.05
Family composition						
Two-parent families	72	75				
Single-parent families	24	25	.98	2	.61	.14
Other compositions	4	0				
Anxiety						
Panic disorder	2.23 (2.01)	11 (6.06)	6.75	27.62	< .01	2.02
Social anxiety disorder	5.92 (3.40)	15.83 (4.99)	8.26	48	< .01	2.38
Separation anxiety	0.50 (0.81)	4.83 (4.43)	4.72	24.43	< .01	1.42
Generalized anxiety	3.88 (1.63)	10.21 (3.31)	8.46	32.96	< .01	2.50
Obsessive compulsive disorder	2.04 (1.82)	7.83 (3.14)	8.05	48	< .01	2.32
Total anxiety symptomatology	14.58 (6.05)	49.71 (16.32)	9.94	28.79	< .01	2.71
SP	4.11 (2.40)	10.00 (2.43)	8.60	48	< .01	2.48
Cardiac measures						
HR	93.99 (11.17)	99.48 (8.42)	1.95	48	.06	.55
SDANN	42.50 (17.13)	44.48 (10.38)	.50	41.69	.62	.19
lnHF	6.26 (0.66)	5.86 (0.78)	1.95	48	.06	−.55
h exponent	0.89 (0.04)	0.92 (0.03)	2.90	48	< .01	.82

Note. Numerical data are presented using the average and standard deviations (between brackets). Categorical variables (gender and family composition) are showed as percentage of cases.

[†] Effect sizes were calculated using the Cramer's ϕ statistic for categorical data and Cohen's *d* statistic for numerical data.

SP = Sensitivity to punishment.

HR = Heart rate (measured in bpm); SDANN = standard deviation of the averages of normal-to-normal beat intervals in all 5-minute segments of the entire recording; lnHF = logarithm of high frequency band power.

Table 2 Pearson's correlations between cardiac measures and psychological variables.

	RCADS PD	SAD	SepAnx	GAD	OCD	Total Anx	SPSRQ-J SP
vmHRV (lnHF)	−.16	-.19	-.01	-.07	-.20	-.16	-.21
Scaling exponents *h*	.26	.42**	.12	.29*	.29*	.35*	.38**

Note. RCADS = Revised Children's Anxiety and Depression Scale; SPSRQ-J = Sensitivity to Punishment and Sensitivity to Reward Questionnaire Junior.

PD = Panic disorder; SAD = Social anxiety disorder; SepAnx = Separation anxiety; GAD = Generalized anxiety disorder; OCD = Obsessive compulsive disorder; Total Anx = Total anxiety symptomatology scale.

SP = Sensitivity to punishment.

vmHRV = Vagally-mediated heart rate variability.

* Two-tailed $p < .05$;

** $p < .01$.

scores, and the sensitivity to punishment scores had a significant positive correlation to the scaling exponents *h*, and therefore the higher the exponents, the higher the anxiety symptoms and the SP scores.

As the *h* values ranged from 0.78 to 0.96, higher exponents were closer to 1 (thus indicating a lower fractal dimension value). If scaling exponents reflect allometric control, then this finding shows that a fractal (or multistability) loss would be associated with anxiety and SP. Therefore, it is not (or not only) the real-time homeostatic control provided by the parasympathetic system that is altered in highly anxious adolescents but the allometric control that depends on the interaction among multiple sources (the parasympathetic and the sympathetic branches of the autonomous nervous system among others) and that operates at multiple time scales (see Ivanov et al., 2004).

The lack of associations between vmHRV and anxiety and SP scores deserves a comment as it appears to be contrary to many findings reported in the last decade. Usually, vmHRV has been estimated from the calculation of the HF band power on short recordings taken in lab conditions (participants seated in resting conditions for five minutes or so). Actually, this is a good way to measure the participants' vagal tone, i.e. the influence of the parasympathetic system on HRV at rest, when participants are not engaged in any school-related activity. This measure, however, may not

be a good way to evaluate vmHRV in everyday life conditions as it encompasses tonic but also phasic (reactive) influences of the parasympathetic system. Therefore, the findings we report here are not contrary but complementary to the vmHRV-related findings, as the latter refer to vagal tone while the former should be seen within the wider context of the parasympathetic system operating during longer periods (1.5 hours) in everyday activities. Other studies with clinical samples of adults and long-term recordings under ecological conditions provided similar findings (Agorastos et al., 2013; Yeragani, Nadella, Hinze, Yeragani, & Jampala, 2000).

Regarding the study limitations, we must discuss the absence of randomized sample selection in a twofold way: high schools which accepted to participate into the study; and participants by themselves. However, we initially selected randomly a collection of 20 high schools across the Mallorca Island. Thirteen high school boards deliberately accepted (and perhaps these were biased someway), and all of the participants that filled out the questionnaires and satisfied the selection criteria participated in the study to match the sample size requirements for conducting the appropriate hypotheses test analyses (see Bornas et al., 2015, for a wider picture of sample selection flow). Future research should incorporate larger samples in order to assign randomized groups to study conditions. On the other hand, one group showed different proportions of participants according to gender (high anxiety group). However, between-group differences in terms of gender were not found in cardiac measures, supporting the lack of influence of that variable in this study. Nevertheless, future research should take into account the gender in the sample selection process.

As to the clinical implications of the findings of the study, allometric scaling exponents may be useful for assessing adolescents at risk for anxiety disorders (i.e. students with high symptomatology but without an anxiety disorder). It is noteworthy to remember that inexpensive recording devices are already available to assess heartbeat properties in daily settings, and that allometric scaling exponents can be easily calculated using any regular spreadsheet (specific Matlab code is available from the corresponding author). On the other hand, diminished allometric control of the heartbeat may reflect a lack of flexibility needed to cope with the anxiety generating demands that occur in school settings throughout any regular school day. Adolescents who are highly sensitive to punishment show inflexible response patterns (e.g. avoidance) when they have to face anxious situations (Bijttebier et al., 2009). We cannot infer any causal relationship between physiological scaling properties and behavioral patterns from this study, but we have found an association between both kinds of inflexibility that should be the focus of further studies. Even so, our study entails the first approach to addressing how adolescent anxiety-related problems are exhibited in ecological contexts considering nonlinear biomarkers.

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References

Agorastos, A., Boel, J. A., Heppner, P. S., Hager, T., Moeller-Bertram, T., Haji, U., Motazedi, A., Yanaqi, M. A., Baker, D. G., & Stiedl, O. (2013). Diminished vagal activity and blunted diurnal variation of heart rate dynamics in posttraumatic stress disorder. *Stress*, *16*, 300–310. http://doi.org/10.3109/10253890.2012.751369

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.

Balle, M., Morillas, A., Tortella-Feliu, M., & Bornas, X. (2015). Heart rate complexity and attentional orienting: A new look at the role of vagal tone. *Nonlinear Dynamics, Psychology, and Life Sciences*, *19*, 101–110.

Balle, M., Tortella-Feliu, M., & Bornas, X. (2013). Distinguishing youths at risk for anxiety disorders from self-reported BIS sensitivity and its psychophysiological concomitants. *International Journal of Psychology*, *48*, 964–977.

Beauchaine, T. P. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology*, *13*, 183–214. http://doi.org/10.1017/S0954579401002012

Beauchaine, T. P., & Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. *International Journal of Psychophysiology*. http://doi.org/10.1016/j.ijpsycho.2015.08.004

Bijttebier, P., Beck, I., Claes, L., & Vandereycken, W. (2009). Gray's Reinforcement Sensitivity Theory as a framework for research on personality-psychopathology associations. *Clinical Psychology Review*, *29*, 421–430. http://doi.org/10.1016/j.cpr.2009.04.002

Bornas, X., Balle, M., de la Torre-Luque, A., Fiol-Veny, A., & Llabrés, J. (2015). Ecological assessment of heart rate complexity: Differences between high- and low-anxious adolescents. *International Journal of Psychophysiology*, *98*, 112–118. http://doi.org/10.1016/j.ijpsycho.2015.07.007

Bornas, X., Llabrés, J., Balle, M., de la Torre-Luque, A., & Fiol-Veny, A. (2014). The use of a secure social network for the study of anxiety in adolescence. *Revista de Psicopatología y Psicología Clínica*, *19*, 171–184. http://dx.doi.org/10.5944/rppc.vol.19.num.3.2014.13899

Bornas, X., Noguera, M., Pincus, D., & Buela-Casal, G. (2014). Emotional inertia: A key to understanding psychotherapy process and outcome. *International Journal of Clinical and Health Psychology*, *14*, 232–239. http://dx.doi.org/10.1016/j.ijchp.2014.03.001

Bravi, A., Longtin, A., & Seely, A. J. E. (2011). Review and classification of variability analysis techniques with clinical applications. *Biomedical Engineering Online*, *10*, 90. http://doi.org/10.1186/1475-925X-10-90

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., & Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: A meta-analysis. *Frontiers in Psychiatry*, *5*, 80. http://doi.org/10.3389/fpsyt.2014.00080

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of DSM-IV anxiety and depression in children: A Revised Child Anxiety and Depression Scale. *Behaviour Research and Therapy*, *38*, 835–855. http://doi.org/10.1016/S0005-7967(99)00130-8

Fritz, C. O., Morris, P. E., & Richler, J. J. (2012). Effect size estimates: Current use, calculations, and interpretation. *Journal of Experimental Psychology: General*, *141*, 2–18. http://doi.org/10.1037/a0024338

Goldberger, A. L. (1996). Non-linear dynamics for clinicians: Chaos theory, fractals, and complexity at the bedside. *The Lancet*, *347*, 1312–1314. http://doi.org/10.1016/S0140-6736(96)90948-4

Goldberger, A. L., Amaral, L. A. N., Glass, L., Hausdorff, J. M., Ivanov, P. C., Mark, R. G., Mietus, J. E., Moody, G. B., Peng, C.-K., & Stanley, H. E. (2000). PhysioBank, PhysioToolkit, and PhysioNet. Components of a new research resource for complex physiologic signals. *Circulation*, *101*, e215–e220.

Goldberger, A. L., Amaral, L. A. N., Hausdorff, J. M., Ivanov, P. C., Peng, C.-K., & Stanley, H. E. (2002). Fractal dynamics in physiology: Alterations with disease and aging. *Proceedings of the National Academy of Sciences*, *99*, 2466–2472. http://doi.org/10.1073/pnas.012579499

Ivanov, P. C., Chen, Z., Hu, K., & Stanley, H. E. (2004). Multiscale aspects of cardiac control. *Physica A: Statistical Mechanics and Its Applications*, *344*, 685–704. http://doi.org/10.1016/j.physa.2004.08.016

Kristensen, H., Oerbeck, B., Torgersen, H. S., Hansen, B. H., & Wyller, V. B. (2014). Somatic symptoms in children with anxiety disorders: An exploratory cross-sectional study of the relationship between subjective and objective measures. *European Child & Adolescent Psychiatry*, *23*, 795–803. http://doi.org/10.1007/s00787-013-0512-9

Lehrer, P., & Eddie, D. (2013). Dynamic processes in regulation and some implications for biofeedback and biobehavioral interventions. *Applied Psychophysiology and Biofeedback*, *38*, 143–155, http://doi.org/10.1007/s10484-013-9217-6.

Mandelbrot, B. (1967). How long is the coast of Britain? Statistical self-similarity and fractional dimension. *Science*, *156*, 636–638.

Montero, I., & León, O. (2007). A guide for naming research studies in Psychology. *International Journal of Clinical and Health Psychology*, *7*, 847–862.

Nardelli, M., Valenza, G., Cristea, I. A., Gentili, C., Cotet, C., David, D., Lanata, A., & Scilingo, E. P. (2015). Characterizing psychological dimensions in non-pathological subjects through autonomic nervous system dynamics. *Frontiers in Computational Neuroscience*, *9*, 37. http://doi.org/10.3389/fncom.2015.00037

Panayiotou, G., Karekla, M., & Panayiotou, M. (2014). Direct and indirect predictors of social anxiety: The role of anxiety sensitivity, behavioral inhibition, experiential avoidance and self-consciousness. *Comprehensive Psychiatry*, *55*, 1875–1882. http://doi.org/10.1016/j.comppsych.2014.08.045

Peng, C.-K., Havlin, S., Stanley, H. E., & Goldberger, A. L. (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos*, *5*, 82–87. http://doi.org/10.1063/1.166141

Porges, S. W. (2001). The polyvagal theory: Phylogenetic substrates of a social nervous system. *International Journal of Psychophysiology*, *42*, 123–146. http://doi.org/10.1016/S0167-8760(01)00162-3

Sandín, B., Valiente, R. M., & Chorot, P. (2009). RCADS: Assessment of anxiety and depressive disorder symptoms in children and adolescents. *Revista de Psicopatología y Psicología Clínica*, *14*, 193–206. http://dx.doi.org/10.5944/rppc.vol.14.num.3.2009.4078

Shaffer, F., McCraty, R., & Zerr, C. L. (2014). A healthy heart is not a metronome: An integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology*, *5*, 1040. http://doi.org/10.3389/fpsyg.2014.01040

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*, 22–33. http://doi.org/10.1016/S0924-9338(99)80239-9

Stadnitski, T. (2012). Measuring fractality. *Frontiers in Physiology*, *3*, 127. http://doi.org/10.3389/fphys.2012.00127

Sturmberg, J. P., Bennett, J. M., Picard, M., & Seely, A. J. E. (2015). The trajectory of life. Decreasing physiological network complexity through changing fractal patterns. *Frontiers in Physiology*, *6*, 169. http://doi.org/10.3389/fphys.2015.00169

Tarvainen, M., Niskanen, J., Lipponen, J. A., Ranta-aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV-heart rate variability analysis software. *Computer Methods and Programs in Biomedicine*, *113*, 210–220.

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders*, *61*, 201–216. http://doi.org/10.1016/S0165-0327(00)00338-4

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews*, *33*, 81–88. http://doi.org/10.1016/j.neubiorev.2008.08.004

Torrubia, R., García-Carrillo, M., Ávila, C., Caseras, X., & Grande, I. (2008). *A junior questionnaire for the measurement of sensitivity to punishment and sensitivity to reward*. Unpublished manuscript, Universitat Autònoma de Barcelona.

West, B. J. (2006). *Where medicine went wrong: rediscovering the path to complexity*. Hackensack. NJ: World Scientific.

West, B. J. (2010). Fractal physiology and the fractional calculus: A perspective. *Frontiers in Physiology*, *1*, 12. http://doi.org/10.3389/fphys.2010.00012

West, G. B., Brown, J. H., & Enquist, B. J. (1999). The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science*, *284*, 1677–1679.

Yeragani, V. K., Nadella, R., Hinze, B., Yeragani, S., & Jampala, V. C. (2000). Nonlinear measures of heart period variability: Decreased measures of symbolic dynamics in patients with panic disorder. Depression and Anxiety. *12*, 67–77. http://doi.org/10.1002/1520-6394(2000)12:2<67::AID-DA2>3.0.CO;2-C

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ORIGINAL CONTRIBUTION

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 analysed. 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 12-month period ($n = 24$) showed attenuated heart rate levels in the stage of maximum stress in comparison to their non-increasing anxious counterparts ($p < 0.05$), as well as a heightened pattern of sample entropy throughout the stress induction ($p < 0.05$). These findings suggest a loss of cardiac flexibility in those adolescents at risk of ADs when confronting an acute stressor.

Keywords Anxiety · Trajectory · Stress · Heart rate variability · Adolescence

Introduction

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

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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 of 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 analyse 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 of ADs), in 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 ($f = 0.25$), $\alpha = 0.05$, $1 - \beta = 0.80$, 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 (Fig. 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.

Psychological assessment

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

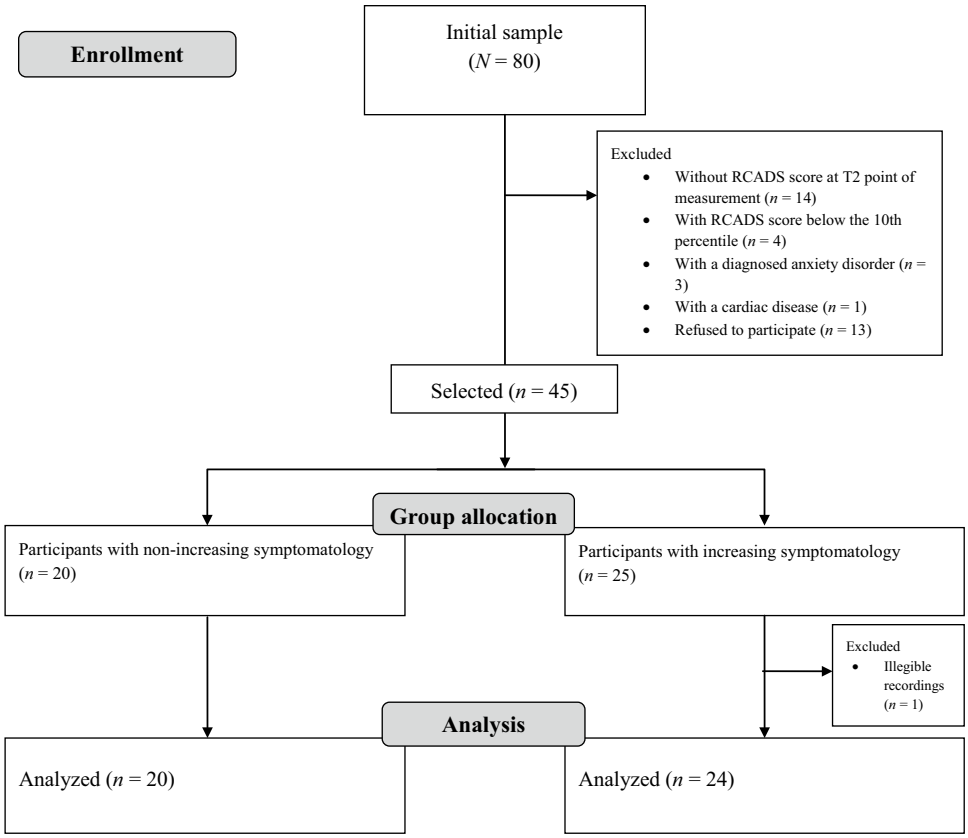

Fig. 1 Flow diagram of sample selection

Table 1 Descriptive features for the study groups

	NIA group ($n = 20$)	IA group ($n = 24$)	χ^2/t^a	*df*	*p*	Effect size[b]
Gender (% boys)	30	41.67	0.64	1	0.42	0.12
Age	14.92 (0.57)	14.85 (0.48)	0.42	38	0.68	0.13
High school area (% urban)	40	33.33	0.21	1	0.65	0.07
Temperament						
EC	4.37 (0.50)	4.11 (0.39)	1.92	42	0.06	0.58
NA	2.85 (0.61)	3.13 (0.50)	−1.66	42	0.07	−0.50
Anxious symptomatology						
T1	32.20 (19.22)	28.21 (15.88)	0.76	42	0.45	0.23
T2	21.90 (15.78)	42.54 (20.45)	−3.69	42	<0.01	−1.13
Change T2–T1[c]	−10.33 (9.82)	14.33 (11.40)	−7.59	42	<0.01	−2.32

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

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

NIA group non-increasing anxiety group, *IA group* increasing anxiety group, *EC* effortful control, *NA* negative affectivity

[a] χ^2 tests for categorical data and *t* tests for numerical variables

[b] Cramer's ϕ for categorical data and Cohen's *d* for numerical data

[c] Difference between measurement point in anxious symptomatology

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 $\alpha = 0.84$ for EC, and $\alpha = 0.65$ for NA).

The Revised Child Anxiety and Depression Scale (RCADS; [40]) is composed of 47 items with a four-point

response scale to evaluate the presence of ADs and depressive symptomatology. The RCADS was administered twice (once and then again after 12 months). We only considered the total anxiety symptomatology scale, which showed adequate reliability at both measurement points (Cronbach's $\alpha = 0.94$ at T1, and $\alpha = 0.97$ at T2; correlation between points, $r = 0.70$, $p < 0.01$).

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 Eq. 1)

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

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 α1 exponents (4–11 heart beats) and the long-term α2 exponents (>11 beats). We only used the α1 exponents due to the length of the study stages. Exponents with values around 0.50 signify uncorrelated data; values from 0.50 to 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 Eq. 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.

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

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 Fig. 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 min under resting conditions (participant should be sitting and relaxed). Afterwards, a researcher explained the task to be performed: participants had to stand up and present themselves, individually. The presentation was 1-min 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-min 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 1-min presentation, participants went back to their seats, and the following 3 min was recorded and considered as the recovery stage.

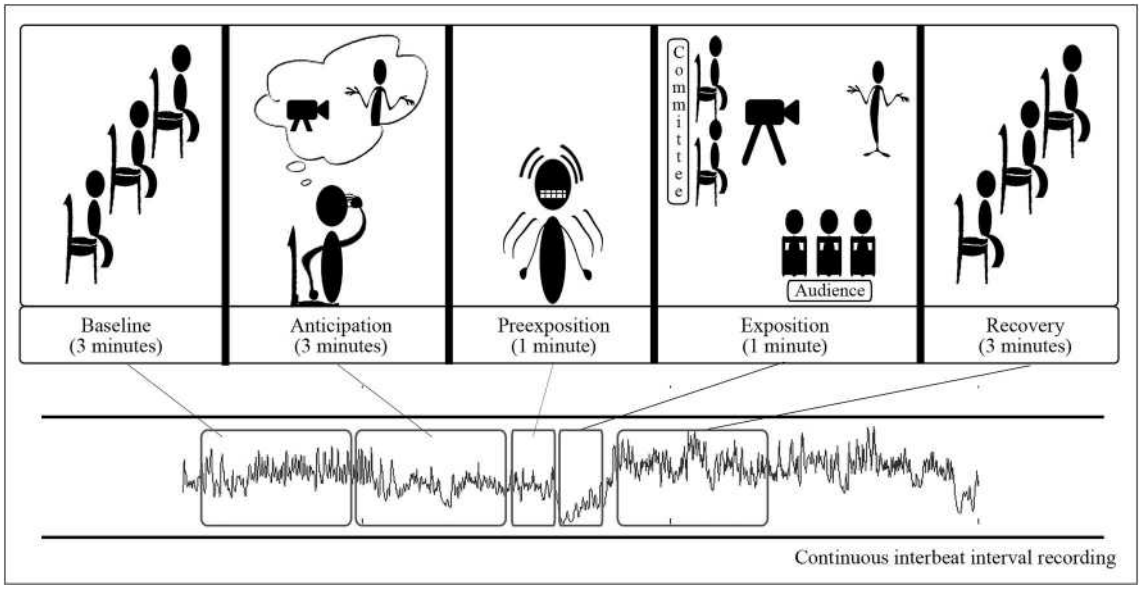

Fig. 2 Summary of the study stages

Data acquisition and preprocessing

IBI time series was 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 min), anticipation stage (3 min), pre-exposition (1 min), exposition (1 min), and recovery (3 min). The pre-exposition stage (i.e. the minute before starting the presentation) was used to control for order effects.

The IBI time series was 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 106,210, 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 α1). 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, $p < 0.01$, $\eta^2_{partial} = 0.20$; and rMSSD, F (4, 160) = 5.01, $p < 0.01$, $\eta^2_{partial} = 0.11$. 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, $p < 0.05$, $\eta^2_{partial} = 0.06$, 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, $p < 0.05$. No other main effects or interaction or covariate effects were found on these dependent variables.

The frequency-domain MANOVA revealed a significant main effect of stage on HF, F (2.95, 115.16) = 3.56, $p < 0.05$, $\eta^2_{partial} = 0.08$; and the DFA exponent, F (4, 156) = 7.12, $p < 0.01$, $\eta^2_{partial} = 0.15$. 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, $p < 0.01$, $\eta^2_{partial} = 0.07$. 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, $p < 0.05$, $\eta^2_{partial} = 0.06$, 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, $p < 0.01$, $\eta^2_{partial} = 0.17$; and a between-group effect was revealed, F (1, 39) = 4.29, $p < 0.05$, $\eta^2_{partial} = 0.10$, signalling higher levels of entropy in IA participants across the study stages (Fig. 3). Post hoc between-group differences were revealed for the anticipation stage, t (42) = −2.31, $p < 0.05$, being marginally significant for the exposition stage, t (42) = −1.80, $p = 0.080$. No other significant post hoc differences were found.

Table 2 Cardiac measures across the study stages

	Stage				
	Baseline	Anticipation	Pre-exposition	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)

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

A main stage effect was found for every cardiac measure ($p < 0.05$). Pairwise comparisons revealed differences between stages ($p < 0.05$, for all comparisons), but not between the baseline and recovery, nor between the anticipation and pre-exposition

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-linear scale, *HF* high-frequency band power (ms^2) in log-linear scale, *DFA α1* short-term scaling exponents, *SampEn* sample entropy, *NIA groups* non-increasing anxiety group, *IA group* increasing anxiety group

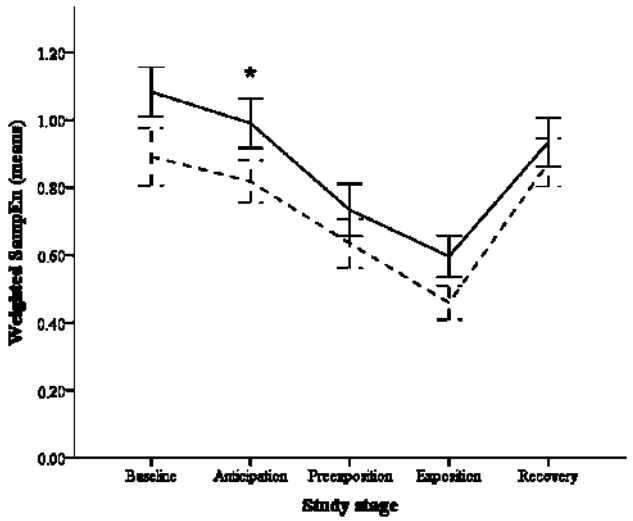

Fig. 3 Weighted sample entropy across the study stages. *Error bars* depict the mean standard error. *Dashed line* non-increasing group, *solid line* increasing anxiety group. *SampEn* sample entropy (weighted by the mean heart rate at baseline stage). *Between-group differences within this stage ($p < 0.05$)

Discussion

Anxiety disorders entail significant degree of mental distress and important sanitary cost. It is highly relevant 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 12-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 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 min 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 of 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.

Compliance with ethical standards

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

References

1. Gunnar MR, Wewerka S, Frenn K, Long JD, Griggs C (2009) Developmental changes in hypothalamus–pituitary–adrenal activity over the transition to adolescence: normative changes and associations with puberty. Dev Psychopathol 21:69–85. doi:10.1017/S0954579409000054
2. Skinner EA, Zimmer-Gembeck MJ (2007) The development of coping. Ann Rev Psychol 58:119–144. doi:10.1146/annurev.psych.58.110405.085705

3. Hollenstein T, Lougheed JP (2013) Beyond storm and stress: typicality, transactions, timing, and temperament to account for adolescent change. Am Psychol 68:444–454. doi:10.1037/a0033586
4. Essau CA, Gabbidon J (2013) Epidemiology, comorbidity and mental health services utilization. In: Essau CA, Ollendick TH (eds) The treatment of childhood and adolescent anxiety. Wiley, Oxford, pp 23–42
5. Essau CA, Lewinsohn PM, Olaya B, Seeley JR (2014) Anxiety disorders in adolescents and psychosocial outcomes at age 30. J Affect Disord 163:125–132. doi:10.1016/j.jad.2013.12.033
6. Gustafsson PE, Gustafsson PA, Ivarsson T, Nelson N (2008) Diurnal cortisol levels and cortisol response in youths with obsessive-compulsive disorder. Neuropsychobiology 57:14–21. doi:10.1159/000123117
7. Schmitz J, Kramer M, Tuschen-Caffier B, Heinrichs N, Blechert J (2011) Restricted autonomic flexibility in children with social phobia. J Child Psychol Psychiatry 52:1203–1211. doi:10.1111/j.1469-7610.2011.02417.x
8. Lyons MD, Huebner ES, Hills KJ (2016) Relations among personality characteristics, environmental events, coping behavior and adolescents' life satisfaction. J Happiness Stud 17:1033–1050. doi:10.1007/s10902-015-9630-z
9. Valiente C, Eisenberg N, Fabes RA, Spinrad TL, Sulik MJ (2014) Coping across the transition to adolescence: evidence of interindividual consistency and mean-level change. J Early Adolesc 35:947–965. doi:10.1177/0272431614548068
10. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G (2014) Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci Biobehav Rev 38:94–124. doi:10.1016/j.neubiorev.2013.11.005
11. Mateo M, Blasco-Lafarga C, Martinez-Navarro I, Guzman JF, Zabala M (2012) Heart rate variability and pre-competitive anxiety in BMX discipline. Eur J Appl Physiol 112:113–123. doi:10.1007/s00421-011-1962-8
12. Vuksanovic V, Gal V (2007) Heart rate variability in mental stress aloud. Med Eng Phys 29:344–349. doi:10.1016/j.medengphy.2006.05.011
13. Kramer M, Seefeldt WL, Heinrichs N, Tuschen-Caffier B, Schmitz J, Wolf OT, Blechert J (2012) Subjective, autonomic, and endocrine reactivity during social stress in children with social phobia. J Abnorm Child Psychol 40:95–104. doi:10.1007/s10802-011-9548-9
14. Klumbies E, Braeuer D, Hoyer J, Kirschbaum C (2014) The reaction to social stress in social phobia: discordance between physiological and subjective parameters. PLoS One 9:e105670. doi:10.1371/journal.pone.0105670
15. Bornas X, Riera A, Tortella-Feliu M, Llabres J (2012) Heart rate variability profiles and exposure therapy treatment outcome in flight phobia. Appl Psychophysiol Bioofeedback 37:53–62. doi:10.1007/s10484-011-9179-5
16. Siess J, Blechert J, Schmitz J (2013) Psychophysiological arousal and biased perception of bodily anxiety symptoms in socially anxious children and adolescents: a systematic review. Eur Child Adolesc Psychiatry 23:127–142. doi:10.1007/s00787-013-0443-5
17. Chatkoff DK, Maier KJ, Klein C (2010) Nonlinear associations between chronic stress and cardiovascular reactivity and recovery. Int J Psychophysiol 77:150–156. doi:10.1016/j.ijpsycho.2010.05.008
18. Chida Y, Hamer M (2008) Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. Psychol Bull 134:829–885. doi:10.1037/a0013342
19. Duncko R, Makatsori A, Fickova E, Selko D, Jezova D (2006) Altered coordination of the neuroendocrine response during psychosocial stress in subjects with high trait anxiety. Prog Neuropsychopharmacol Biol Psychiatry 30:1058–1066. doi:10.1016/j.pnpbp.2006.04.002
20. Schmitz J, Ruschen-Caffier B, Wilhelm FH, Blechert J (2013) Taking a closer look: autonomic dysregulation in socially anxious children. Eur Child Adolesc Psychiatry 22:631–640. doi:10.1007/s00787-013-0405-y
21. Beauchaine TP, Thayer JF (2015) Heart rate variability as a transdiagnostic biomarker of psychopathology. Int J Psychophysiol 98:338–350. doi:10.1016/j.ijpsycho.2015.08.004
22. Cavanagh JF, Allen JJB (2008) Multiple aspects of the stress response under social evaluative threat: an electrophysiological investigation. Psychoneuroendocrinol 33:41–53
23. Gunnar M, Quevedo K (2007) The neurobiology of stress and development. Ann Rev Psychol 58:145–173. doi:10.1146/annurev.psych.58.110405.085605
24. Talge NN, Donzella B, Gunnar MR (2008) Fearful temperament and stress reactivity among preschool-aged children. Infant Child Dev 17:427–445. doi:10.1002/icd.585
25. Miers AC, Blote AW, de Rooij M, Bolhorst CL, Westenberg PM (2013) Trajectories of social anxiety during adolescence and relations with cognition, social competence, and temperament. J Abnorm Child Psychol 41:97–110. doi:10.1007/s10802-012-9651-6
26. Insel T (2014) The NIMH Research Domain Criteria (RDoC) project: precision medicine for psychiatry. Am J Psychiatry 171:395–397. doi:10.1176/appi.ajp.2014.14020138
27. Insel T, Cuthbert B, Heinssen R et al (2010) Research Domain Criteria (RDoC): toward a new classification framework for research on m ental disorders. Am J Psychiatry 167:748–751. doi:10.1176/appi.ajp.2010.09091379
28. Okasha A (2009) Would the use of dimensions instead of categories remove problems related to subthreshold disorders? Eur Arch Psychiatry Clin Neurosci 259:S129–S133. doi:10.1007/s00406-009-0052-y
29. Shankman SA, Klein DN, Lewinsohn PM, Seeley JR, Small JW (2008) Family study of subthreshold psychopathology in a community sample. Psychol Med 38:187–198. doi:10.1111/j.1469-7610.2009.02117.x
30. Hale WW III, Raaijmakers Q, Muris P, van Hoof A, Meeus W (2008) Developmental trajectories of adolescent anxiety disorder symptoms: a 5-year prospective community study. J Am Acad Child Adolesc Psychiatry 47:556–564. doi:10.1097/CHI.0b013e3181676583
31. Sportel BE, Nauta MH, de Hullu E, de Jong PJ, Hartman CA (2011) Behavioral inhibition and attentional control in adolescents: robust relationships with anxiety and depression. J Child Fam Stud 20:149–156. doi:10.1007/s10826-010-9435-y
32. McLaughlin KA, King K (2015) Developmental trajectories of anxiety and depression in early adolescence. J Abnorm Child Psychol 43:311–323. doi:10.1007/s10802-014-9898-1
33. Morin AJS, Maiano C, Nagensgast B, Marsh HW, Morizot J, Janosz M (2011) General growth mixture analysis of adolescents' developmental trajectories of anxiety: the impact of untested invariance assumptions on substantive interpretations. Struct Equ Modeling 18:613–648. doi:10.1080/10705511.2011.607714
34. Shankman SA, Lewinsohn PM, Klein DN, Small JW, Seeley JR, Altman SE (2009) Subthreshold conditions as precursors for full syndrome disorders: a 15-year longitudinal study of multiple diagnostic classes. J Child Psychol Psychiatry 50:1485–1494. doi:10.1111/j.1469-7610.2009.02117.x
35. Faul F, Erdfelder E, Lang AG, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175–191. doi:10.3758/BF03193146

36. Bornas X, Llabres J, Balle M, de la Torre-Luque A, Fiol-Veny A (2014) The use of a secure social network for the study of anxiety in adolescence. Revista Psicopatologia Psicologia Clinica 19:171–184. doi:10.5944/rppc.vol.19.num.3.2014.13899
37. Ellis LK, Rothbart MK (2001) Revision of the Early Adolescent Temperament Questionnaire. Poster session presented at the Biennial Meeting of the Society for Research in Child Development, Minneapolis
38. Rothbart MK (2007) Temperament, development, and personality. Curr Dir Psychol Sci 16:207–212. doi:10.1111/j.1467-8721.2007.00505.x
39. Rapee RM, Coplan RJ (2010) Conceptual relations between anxiety disorder and fearful temperament. New Dir Child Adolesc Dev 127:17–31. doi:10.1002/cd.260
40. Chorpita BF, Yim L, Moffitt C, Umemoto LA, Francis SE (2000) Assessment of symptoms of DSM-IV anxiety and depression in children: a revised child anxiety and depression scale. Behav Res Ther 38:835–855. doi:10.1016/S0005-7967(99)00130-8
41. Sheehan DV, Lecrubier Y, Harnett-Sheehan K et al (1998) The mini international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview. J Clin Psychiatry 59:22–33
42. Camm AJ, Malik M, Bigger JT Jr et al (1996) Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Eur Heart J 17:354–381
43. Peng CK, Havlin S, Stanley HE, Goldberger AL (1995) Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos 5:82–87
44. Richman JA, Moorman JR (2000) Physiological time-series analysis using approximate entropy and sample entropy. Am J Physiol Heart Circ Physiol 278:H2039–H2049
45. Von Dawans B, Kirschbaum C, Heinrichs M (2011) The Trier Social Stress Test for Groups (TSST-G): a new research tool for controlled simultaneous social stress exposure in a group format. Psychoneuroendocrinol 36:514–522. doi:10.1016/j.psyneuen.2010.08.004
46. Kirschbaum C (2010) Trier social stress test. In: Stolerman IP (ed) Encyclopedia of Psychopharmacology (chapter ID: 0001093214). Springer-Verlag, Berlin
47. Tarvainen M, Niskanen J, Lipponen JA, Ranta-aho PO, Karjalainen PA (2014) Kubios HRV-heart rate variability analysis software. Comput Methods Programs Biomed 113:210–220
48. Young H, Benton D (2015) We should be using nonlinear indices when relating heart-rate dynamics to cognition and mood. Sci Rep 5:16619. doi:10.1038/srep16619
49. Billman GE (2013) The effect of heart rate on the heart rate variability response to autonomic interventions. Front Physiol 4:222. doi:10.3389/fphys.2013.00222
50. Sacha J (2013) Why should one normalize heart rate variability with respect to average heart rate. Front Physiol 4:306. doi:10.3389/fphys.2013.00306
51. Senn S (2006) Change from baseline and analysis of covariance revisited. Stat Med 25:4334–4344. doi:10.1002/sim.2682
52. Hollenstein T, Lichtwarck-Aschoff A, Potworowski G (2013) A model of socioemotional flexibility at three time scales. Emot Rev 5:397–405. doi:10.1177/1754073913484181
53. Thayer JF, Ahs F, Fredrikson M, Sollers JJ III, Wager TD (2012) A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. Neurosci Biobehav Rev 36:747–756. doi:10.1016/j.neubiorev.2011.11.009
54. Anderson ER, Hope DA (2009) The relationship among social phobia, objective and perceived physiological reactivity, and anxiety sensitivity in an adolescent population. J Anxiety Disord 23:18–26. doi:10.1016/j.janxdis.2008.03.011
55. Bornas X (2016) Mesures quantitatives de la complexitat (quantitative measures of complexity). Psicopatologia i caos (Psychopatology and chaos), 2nd edn. Bubok, Madrid, pp 127–152
56. Friedman BH (2007) An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. Biol Psychol 74:185–199. doi:10.1016/j.biopsycho.2005.08.009
57. De la Torre-Luque A, Caparros-Gonzalez RA, Bastard T, Vico FJ, Buela-Casal G (2016) Acute stress recovery through listening to Melomics relaxing music: a randomized controlled trial. Nord J Music Ther. doi:10.1080/08098131.2015.1131186
58. Williamon A, Aufegger L, Wasley D, Looney D, Mandic DP (2013) Complexity of physiological responses decreases in high-stress musical performance. J R Soc Interface 10:20130719. doi:10.1098/rsif.2013.0719
59. Porta A, Gnecchi-Ruscone T, Tobaldini E, Guzzetti S, Furlan R, Montano N (2007) Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. J Appl Physiol 103:1143–1149. doi:10.1152/japplphysiol.00293.2007
60. Porta A, Guzzetti S, Furlan R, Gnecchi-Ruscone T, Montano N, Malliani A (2007) Complexity and non linearity in short-term heart period variability: comparison of methods based on local non linear prediction. IEEE Trans Biomed Eng 54:94–106. doi:10.1109/TBME.2006.883789
61. De la Torre-Luque A, Fiol-Veny A, Balle M, Bornas X (2016) Heartbeat scaling in early adolescents: its association with anxiety symptoms and sensitivity to punishment. Int J Clin Health Psychol 16:287–294. doi:10.1016/j.ijchp.2016.04.002
62. De la Torre-Luque A, Bornas X, Balle M, Fiol-Veny A (2016) Complexity and nonlinear biomarkers in emotional disorders: a meta-analytic study. Neurosci Biobehav Rev 68:410–422. doi:10.1016/j.neubiorev.2016.05.023
63. Chu BC (2012) Translating transdiagnostic approaches to children and adolescents. Cogn Behav Pract 19:1–4. doi:10.1016/j.cbpra.2011.06.003
64. Essau CA, Olaya B, Sasagawa S, Pithia J, Bray D, Ollendick TH (2014) Integrating video-feedback and cognitive preparation, social skills training and behavioural activation in a cognitive behavioural therapy in the treatment of childhood anxiety. J Affect Disord 167:261–267. doi:10.1016/j.jad.2014.05.056
65. Lehrer PM, Gevirtz R (2014) Heart rate variability biofeedback: how and why does it work? Front Psychol 5:PMC4104929. doi:10.3389/fpsyg.2014.00756

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BRIEF REPORT

Trajectories of anxiety symptoms in adolescents: Testing the model of emotional inertia

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KEYWORDS
Trajectory;
Anxiety;
Emotional inertia;
Treatment energy;
Ex post facto study

Abstract
Background/Objective: Two predictions derived from a recently introduced model of psychotherapy outcome were tested, assuming the dynamical relationship between the individual's emotional trajectory and the force of intervention necessary to change this trajectory: (a) only a high intensity treatment would succeed to lower the increasing trajectory of anxiety, and (b) high as well as low intensity treatments would equivalently lower the non-increasing trajectory of anxiety. *Methods*: Seventy-four adolescents (58.40% girls; $M = 14.65$ years, $SD = 0.53$) were randomly assigned to a high intensity treatment condition, a low intensity treatment condition, or a waiting list condition. *Results:* Only the high intensity treatment reduced the anxiety when participants showed an increasing trajectory ($p < .01$). None of the treatments reduced anxiety when a previously non-increasing trajectory was shown. *Conclusions:* These findings support the theoretical predictions and underscore the need to consider not only how severe the anxiety is but also the time course of anxiety in applied treatment settings.

PALABRAS CLAVE
Trayectoria;
ansiedad;
inercia emocional;
energía del tratamiento;
estudio ex post facto

Trayectoria de la sintomatología ansiosa en adolescentes: poniendo a prueba el modelo de la inercia emocional

Resumen
Introducción/Objetivos: Este informe breve tiene por objetivo poner a prueba dos predicciones derivadas de un reciente modelo sobre los resultados en psicoterapia: (a) solo un tratamiento de alta intensidad sería capaz de cambiar de dirección una trayectoria ascendente de ansiedad, y (b) tanto un tratamiento de alta como de baja intensidad podrían influir en una trayectoria no-ascendente de ansiedad. *Método*: Setenta y cuatro adolescentes (58,40% chicas; $M = 14,65$ años,

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DT = 0,53) fueron asignados aleatoriamente a una de estas condiciones: tratamiento de alta intensidad, tratamiento de baja intensidad o lista de espera. *Resultados*: Solo la aplicación del tratamiento de alta intensidad permitió reducir los niveles de ansiedad en los adolescentes con trayectoria creciente de dicha sintomatología ($p < 0,01$). Además, ambos tratamientos redujeron de forma equivalente la sintomatología en individuos con trayectoria no ascendente de ansiedad. *Conclusiones*: Estos resultados apoyan las predicciones teóricas propuestas y subrayan la necesidad de considerar no solamente la magnitud de la sintomatología ansiosa sino también su curso temporal, en contextos clínicos.

In this brief report we used unpublished data from a treatment study to statistically test two main hypotheses derived from the theoretical model on therapy outcomes introduced by Bornas, Noguera, Pincus, and Buela-Casal (2014). The treatment study was carried out within a longitudinal research project (see Bornas, Llabres, Balle, de la Torre-Luque, & Fiol-Veny, 2014; de la Torre-Luque, Fiol-Veny, Balle, & Bornas, 2016) focused on the trajectories of anxious symptomatology in adolescents. According to that model, treatment outcome depends upon the interaction between the emotional inertia (or resistance to change, Kuppens, Oravecz, & Tuerlinckx, 2010; see also Kuppens, Allen, & Sheeber, 2010) and the treatment's energy (the force a treatment has to overcome inertia). In Physics, inertia is formally defined as ''the property of matter by which it retains [...] its velocity along a straight line so long as it is not acted upon by an external force''. Similarly, emotional inertia would be the property of human emotion by which it retains its course so long as it is not acted upon by an external (e.g. environmental or cognitive) force. One core statement of the theoretical model is that ''the stronger the pull is toward a negative attractor region (...) the more power that is needed to return some flexibility to the system'' (Bornas, Noguera et al., 2014, p. 235). Much like more energy is required to down a balloon that is ascending, more forceful treatments are required to down the anxiety when it is increasing.

Based on this model two testable predictions were made. First, only a high intensity treatment would succeed to lower the increasing (or ascending) trajectory of anxiety, since the pull toward a negative attractor region is stronger when anxiety increases; the change induced by a low intensity treatment would not be significant/meaningful. Second, high as well as low intensity treatments would equivalently lower the non-increasing trajectory of anxiety.

Anxiety trajectories were determined from two assessment points (T1 and T2) with a 6-month interval between them. Participants whose anxiety scores were higher at the second point were labeled as 'increasing anxiety' (IA) adolescents. The rest of them were labeled as 'non-increasing anxiety' (NIA) adolescents. There is no robust classification of psychological treatments based on its energy but some guidelines were proposed by the British National Institute for Health and Care Excellence, NICE (see, for instance, National Institute for Health and Care Excellence, NICE, 2011, pp. 95-96 and pp. 135-137). Likewise, clinicians would probably agree that evidence-based treatments have more energy and clearer protocols (e.g., exposure in Taboas, Ojserkis, & McKay, 2015) than psychoeducation or self-help strategies (see Bornas, Noguera et al., 2014). Treatments in this study were chosen based on these criteria.

Methods

Participants

Seventy-four Spanish adolescents (58.40% girls; $M = 14.65$ years, $SD = 0.53$) participating into the TrAns study were randomly assigned to one of these three conditions: treatment ($n = 27$, 48.27% with increasing anxiety), placebo ($n = 23$, 56.52% with increasing anxiety), and waiting list (WL, $n = 24$, 50% with increasing anxiety). No significant differences were found regarding gender, age and anxiety at the pre-treatment assessment. When the trajectory of anxiety was taken into account, six groups were made up (see Table 1) and submitted to statistical analysis.

Measures

The Revised Child Anxiety and Depression Scale (RCADS; Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000) is a 47-item self-report questionnaire, to evaluate symptomatology of anxiety disorders (separation anxiety disorder, social phobia, generalized anxiety disorder, panic disorder, obsessive-compulsive disorder) and major depressive disorder. Moreover, an overall scale as a total level of anxiety symptoms can be obtained. The internal consistency of the overall anxiety scale was ranged from $\alpha = .94$ and $\alpha = .95$ within our sample, across assessments.

Procedure

The University Bioethics Committee approved all the study procedures, and participants and their parents/tutors provided written consent.

Table 1 Descriptive features for the study groups.

	SSL		WL		SW	
	NIA group	IA group	NIA group	IA group	NIA group	IA group
n	15	12	11	13	10	13
Gender (% boys)	52.63	50	45.45	29.41	40	38.46
Age	14.76 (0.42)	14.60 (0.34)	14.67 (0.53)	14.39 (0.44)	14.67 (0.70)	14.68 (0.61)
Anxiety						
T1	23.68 (17.97)	20.81 (18.57)	30.25 (15.87)	26.29 (19.48)	37.33 (17.68)	31.69 (13.46)
T2	18.16 (16.78)	29.56 (18.94)	23.75 (13.18)	34.88 (21.21)	26.25 (18.26)	38.15 (15.98)
T3	14.27 (13.88)	18.29 (18.14)	25.36 (14.29)	28.79 (21.92)	25.30 (20.22)	35.54 (17.09)

Note. Mean and standard deviations (between brackets) displayed for numerical data, and percentage of cases for gender.
SSL = Treatment group; WL = Waiting-list group; SW = Placebo group; NIA group = Non-increasing anxiety group; IA group = Increasing anxiety group. Anxiety was measured by the RCADS total anxiety scale (measured in 6-month intervals from T1 to T3).

In accordance with the TrAns project schedule, adolescents' anxiety was assessed every six months (T1, T2 or pre-treatment, and T3 or post-treatment). The treatment group received the Super-AD programme, that is the Super Skills for Life (SSL; Essau & Ollendick, 2013) version for Spanish adolescents (de la Torre-Luque, Essau, Fiol-Veny, Balle, & Bornas, 2015). Super-AD constitutes a transdiagnostic preventive intervention focused on averting the onset of internalising syndromes. It consists of eight cognitive-behavioral group sessions. An 8-session group School work (SW) programme was delivered in the placebo group (adolescents did their homework with supervision and school habits and study skills were strengthened). Participants in the WL group followed the regular school activities and received no intervention.

Analytic strategy

RCADS scores were transformed into a loglineal scale to preserve normalized distributions. A mixed repeated-measure ANOVA with one three-level factor Time (T1, T2, T3) and one six-level factor Condition (IA_SSL, IA_SW, IA_WL, NIA_SSL, NIA_SW, and NIA_WL) was performed to evaluate within- as well as between-group differences in the RCADS total scores. The anxiety scores from the WL groups along the three assessment points allowed for observing the maturational trajectory of anxiety. Bonferroni adjusted post hoc tests were used to test for pairwise comparisons.

Results

A significant interaction effect Time x Group, F (10, 136) = 6.13, $p < .001$, $\eta^2_{partial} = .31$, was revealed by the ANOVA, as well as a significant main effect of Time, F (2, 136) = 17.76, $p < .001$, $\eta^2_{partial} = .21$. No significant main effect of Group was found ($p > .10$). The IA groups did not differ in anxiety scores at any assessment time (see Figure 1). Each group showed a significant change in anxious symptomatology scores from T1 to T2. To test the specific predictions derived from the theoretical model we looked at the differences from T2 to T3 in each group. The IA_SSL group was the only one showing a large decrease in anxiety scores from pre- to post-treatment, with a Bonferroni-adjusted t (11) = 5.32, $p < .001$, $d = 1.29$. As to the maturational trajectory shown by the WL groups, there was not a decrease from T1 to T3 in the scores of the NIA_WL group ($p > .05$) whereas the NIA_SSL and the NIA_SW groups showed significant decreases in their anxiety scores, Bonferroni-adjusted t (14) = 4.66, $p < .001$, $d = 1.12$, and t (9) = 3.28, $p = .009$, $d = 0.53$, respectively. Changes in the IA groups from T1 to T3 were not significant (see Figure 1).

Discussion

According to a recently presented model of psychotherapy outcome (Bornas, Noguera et al., 2014) only forceful treatments can successfully overcome the patient's emotional inertia when the trajectory of anxiety is ascending/increasing. Low intensity treatments may succeed if the trajectory is not increasing. These predictions were statistically tested using data from a larger study where three groups of adolescents received three treatment conditions (SSL as the high intensity treatment, SW as the low intensity treatment, and WL or no treatment). Based on the individual anxiety trajectory (ascending or non-increasing) each group was divided into two subgroups (IA or NIA). This classification was validated by the differences in anxiety scores from T1 to T2 in all the groups. Anxiety scores from the WL groups along T1, T2, and T3 were taken as the best approach to the maturational trajectory of the anxious symptomatology scores.

The first prediction was confirmed by the results of the ANOVA. Only the high intensity treatment (SSL) largely reduced the anxiety symptomatology from pre- to post-treatment of participants with a previous increasing trajectory. Further, the energy of the SW treatment should be considered very low since the anxiety scores from the IA_WL group did not change either. In other words, the SW treatment was not able to change the maturational time course of anxiety. It is worth to say that the anxiety scores from the three IA groups at T3 (post-treatment) were statistically equivalent, i.e. all groups showed similar anxiety scores. This is not contrary to our hypothesis (the SSL did decrease the anxiety whereas the other two condition did not), but it shows that the SSL package is not a very forceful treatment. Indeed, the SSL treatment has a preventive character and it is not designed to treat severe anxiety disorders. This finding confirms the need to use more intense

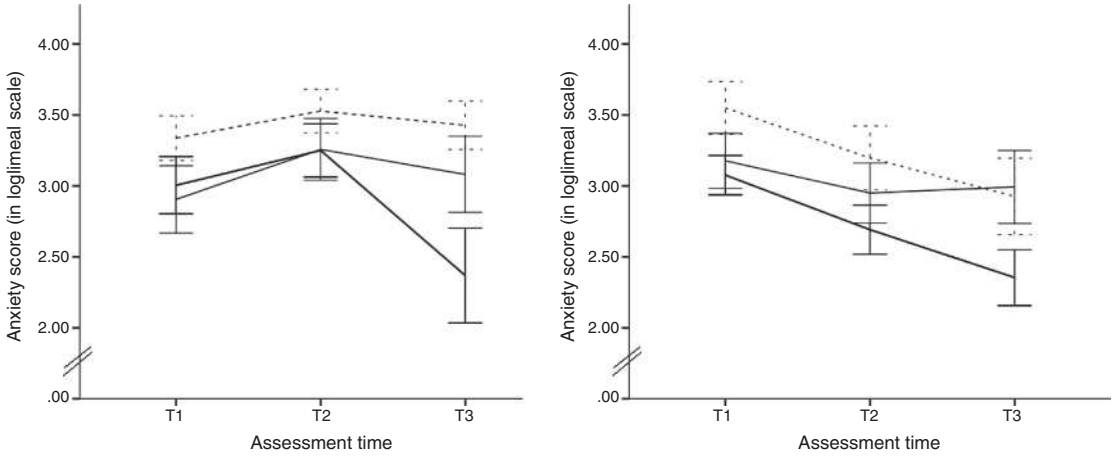

Figure 1 The trajectories of anxious symptomatology for the increased anxiety (IA) groups (left) and the non-increased anxiety (NIA) groups (right) across the three assessment times.
Note. Anxiety scores, displayed under a loglineal transformation, from the treatment (SSL) groups (dark lines), the waiting-list groups (solid grey lines), and the placebo (SW) groups (dashed grey lines).
Error bars depict the standard error of the mean.

treatments to overcome the emotional inertia of very anxious adolescents as well as adolescents suffering from a diagnosed anxiety disorder.

The second prediction stated that the effect of high and low intensity treatments when the anxiety trajectory was non-increasing would equivalently deviate the non-increasing trajectory of anxiety. The results partly confirmed this prediction. Neither the SSL nor the SW conditions significantly lowered the anxiety scores of the NIA groups from T2 to T3, and therefore their effects were ''equivalent''. We are aware, however, that the expected effect of any psychological intervention is not the ''unchange'' of the target (in this study, the anxiety scores). If we look at the changes in the NIA_WL group we see that its anxiety scores remained at the same level from T1 to T3. Therefore, the maturational course of anxiety along this period or time (and at this age) tends to stabilize. Since the NIA groups that received psychological treatment reduced the anxiety scores from T1 to T3, we can speculate that both of them had at least a weak impact on the maturational trajectory of anxiety. On the other hand, a floor effect may help to understand this result. Obviously, anxiety is not expected to decrease forever, and therefore the decreasing trajectory of the NIA groups should stabilize at some moment (i.e. when it reaches the ''floor''). It is not reasonable, therefore, to expect that any treatment, disregarding its energy, will reduce the anxiety beyond this floor. The floor effect may be helpful also to understand why the SSL treatment reduced the anxiety of the IA group but it did not reduce the scores of the NIA group. The IA group anxiety at T2 was far from the floor and could be much reduced whereas the anxiety level in the NIA group at T2 was too close to the floor.

To sum up, this brief report underscores the need to consider (when possible) not only how severe the anxiety is but also the time course of anxiety. In this way, the effects of treatments can be explained not only by their energy (e.g. degree of protocolization and scheduling, use of tested techniques, etc.) but also by their impact on systems (adolescents' emotions) that move and follow specific trajectories across time.

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References

Bornas, X., Llabres, J., Balle, M., de la Torre-Luque, A., & Fiol-Veny, A. (2014). The use of a secure social network for the study of anxiety in adolescence. *Revista de Psicopatología y Psicología Clínica*, *19*, 171–184. http://dx.doi.org/10.5944/rppc.vol.19.num.3.2014.13899

Bornas, X., Noguera, M., Pincus, D., & Buela-Casal, G. (2014). Emotional inertia: A key to understanding psychotherapy process and outcome. *International Journal of Clinical and Health Psychology*, *14*, 232–239. http://dx.doi.org/10.1016/j.ijchp.2014.03.001

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of DSM-IV anxiety and depression in children: A revised child anxiety and depression scale. *Behaviour Research and Therapy*, *38*, 835–855. http://dx.doi.org/10.1016/S0005-7967(99)00130-8

De la Torre-Luque, A., Essau, C., Fiol-Veny, A., Balle, M., & Bornas, X. (2015, December). *Super Skills for Life for Spanish adolescents: The Super-AD programme*. Poster session presented at the European Symposium on Adolescent Research, London.

De la Torre-Luque, A., Fiol-Veny, A., Balle, M., & Bornas, X. (2016). Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment. *International Journal of Clinical and Health Psychology*, *16*, 287–294. http://dx.doi.org/10.1016/j.ijchp.2016.04.002

Essau, C., & Ollendick, T. (2013). *The Super Skills for Life programme*. London: University of Roehampton.

Kuppens, P., Allen, N. B., & Sheeber, L. B. (2010). Emotional inertia and psychological maladjustment. *Psychological Science*, *21*, 984–991. http://dx.doi.org/10.1177/0956797610372634

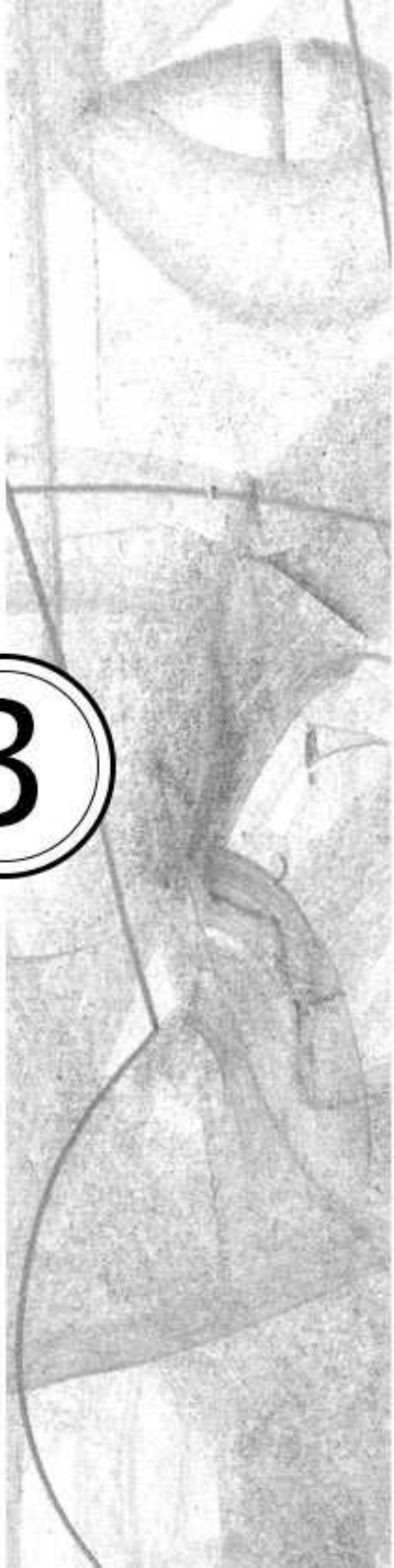
Kuppens, P., Oravecz, Z., & Tuerlinckx, F. (2010). Feelings change: Accounting for individual differences in the temporal dynamics of affect. *Journal of Personality and Social Psychology*, *99*, 1042–1060. http://dx.doi.org/10.1037/a0020962

National Institute for Health and Care Excellence, NICE (2011). *Generalised anxiety disorder in adults: management in primary, secondary and community care*. London: British Psychological Society.

Taboas, W., Ojserkis, R., & McKay, D. (2015). Change in disgust reactions following cognitive-behavioral therapy for childhood anxiety disorders. *International Journal of Clinical and Health Psychology*, *15*, 1–7. http://dx.doi.org/10.1016/j.ijchp.2014.06.002

General Discussion and Conclusions

3

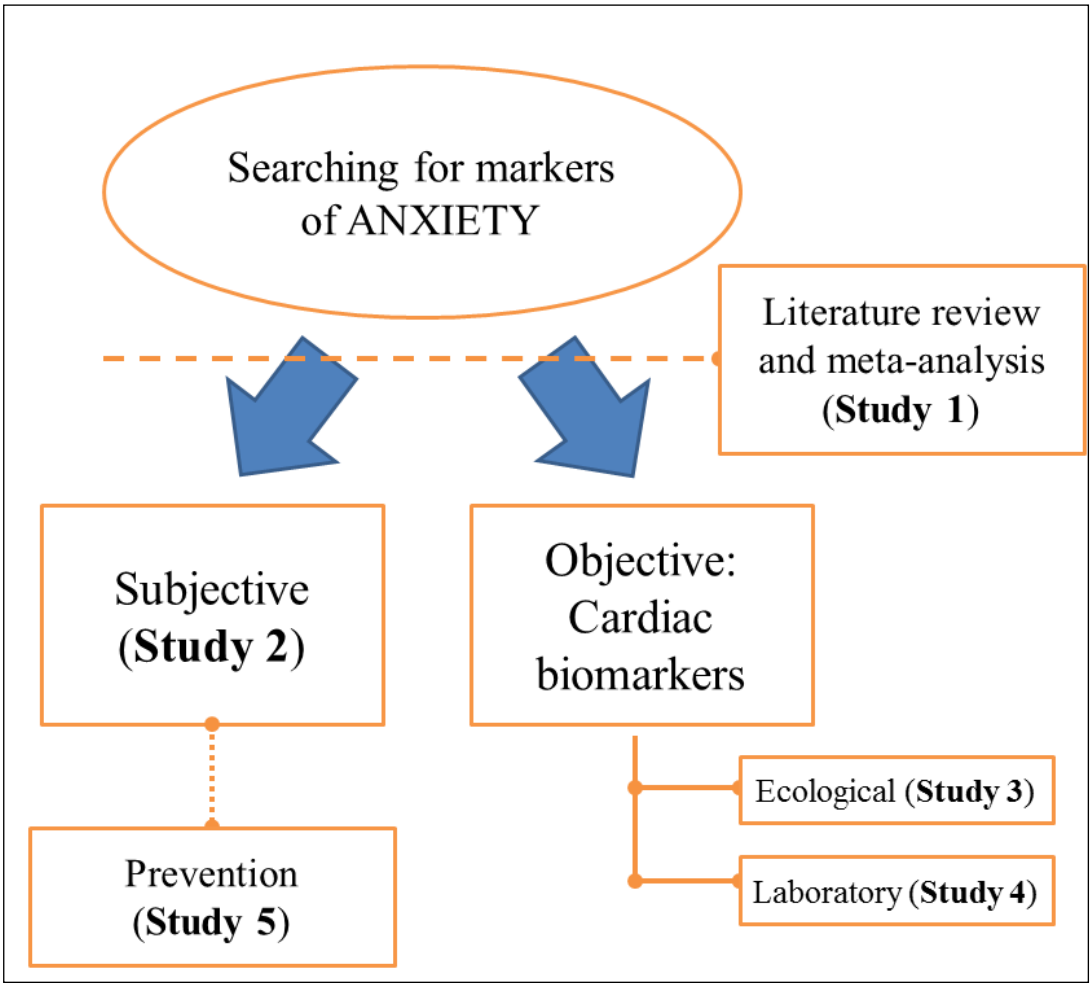


This doctoral dissertation essentially aims at overcoming some major shortcomings in the field of adolescent anxiety. For this reason, five studies were conducted and some robust empirical evidence in the field was obtained. All the study protocols were designed in keeping with the principles of a flexible approach for psychopathology: the RDoC initiative. Thus, the traditional, categorical method of understanding mental distress and psychopathology (based on traditional diagnostic manuals) was taken as a starting point (in fact, Study 1 was a meta-analysis solely covering studies with full-blown disordered individuals), but approaching different conditions along the continuum of anxiety across the studies (e.g., community samples, individuals with rising trajectories of symptoms over time, or individuals with high symptomatology at a specific assessment point). Furthermore, and also in line with RDoC recommendations, anxiety was looked at from multiple levels of analysis (e.g., behavioral, physiological and self-reported) and covering different domains (i.e., the negative valence systems domain and the arousal and regulatory systems domain) across studies.

As stated above, an interest in groundbreaking but reliable indicators of anxiety was the basis of this doctoral dissertation (see Figure 3). Accordingly, it adhered to assumptions from the DST framework across the studies. Many previous findings have endorsed how useful the DST-related measures may be in the physiology and emotional field (Fiol-Veny et al., 2017; Chialvo, 2010; Freeman, 1999; Ivanov et al., 2004; West, 2006; Yeragani et al., 2000): the discovery of the complex and multistable nature of brain dynamics and heartbeat, the self-similarity properties of daily mood, the built-in long-time memory process involved in the functioning of physiological systems, etc. This is why Study 1 aimed at gathering empirical evidence on how nonlinear measures might be reliable markers of emotional disorders (anxiety disorders, depressive and bipolar disorders). To do this, a meta-analytic approach was taken, systematically reviewing scientific empirical papers to obtain integrated conclusions from existing literature. As a result, the overall hypothesis in this study (disordered human systems would show lower complexity in their functioning than healthy systems) was endorsed.

Nonetheless, some clarifications should be added, with the occasional loss of some ordered complexity (e.g., the more irregular, dispersed electrical activity in the brains of depressive individuals) being proved as significant finding in human systems of individuals with emotional disorders.

Figure 3. Visual summary of the thesis' main goals and related studies.



Other outstanding conclusions were discussed in this study but, bearing in mind the thesis' overall purpose, it is worth noting that some relevant biomarkers were identified for individuals with ADs. First, the loss of ordered complexity in the

respiratory system seemed to be a clear and accurate indicator of panic disorder, as some nonlinear measures showed (informational measures: entropy-related). Second, a loss of complexity in brain and cardiac dynamics also turned out to be at the basis of human anxiety disordered systems, especially considering measures from the invariance domain (e.g., largest Lyapunov exponent or fractal dimension measures).

Study 1 was the starting point for the doctoral dissertation as it involved a thorough literature review. Studies 2, 3 and 4 were more focused on identifying concrete and specific markers for maladaptive anxiety in early and middle adolescence. Study 2 focused on describing subjective markers of anxiety. Specifically, it intended to examine how anxiety evolved from early to middle adolescence. Moreover, there was also an interest in examining the role of maturing, temperament under maturation (towards the adult personality) and gender in anxiety development. This longitudinal study involved monitoring a community sample of more than 800 adolescents every six months from age 12-13 to age 15-16. Furthermore, and in line with the Flex3 model (Hollenstein et al., 2013), it studied two important items from two different scales: temperament (macro scale) and anxiety symptomatology (meso scale). Finally, a mixed modelling approach was taken: this enabled consideration of the overall trend of anxiety symptom manifestation across assessments as well as its potential person-specific trajectories.

Findings derived from Study 2 highlighted a curvilinear, descending course of anxiety from early to middle adolescence, although some classes of individuals with varying trajectories were found. Moreover, anxiety tended to be expressed through adopting specific forms over time (e.g., panic anxiety, social phobia, etc.). In addition, it is important to state that social phobia symptomatology reached peaks of clinical symptomatology across assessment points in over 35% of participants. This finding supports the hypothesis that middle adolescence is a sensitive period for social phobia (Copeland et al., 2014; Kessler et al., 2012; Paus et al., 2008). Finally, a mention must be made of the risk factors (FT and AC as temperamental factors and gender) considered in this study. Traditionally, the temperamental risk profile for ADs involved exhibiting low AC and high FT (see, for instance, Sportel et al., 2011; Susa, Pitica,

Benga, & Miclea, 2012). However, a longitudinal, developmental perspective of these factors has traditionally been overlooked. Study 2 pointed out the importance of this risk profile in early adolescence on anxiety development throughout middle adolescence, regardless of the risk factors possibly reaching normative levels afterward.

In the search for markers of adolescent anxiety, Study 3 and Study 4 focused on cardiac biomarkers, showing some complementary evidence. Study 3 aimed at gathering some evidence on the differential cardiac function of adolescents with high anxious symptomatology in comparison to individuals with low levels. Moreover, it aimed to study the associations of different potential biomarkers (HF power and allometric exponent *h*) with anxiety symptoms and an FT-related trait (sensitivity to punishment). Adolescents were monitored within their ecological context, i.e., on a regular school day. Moreover, recordings were longer than those traditionally found in the existing literature (short recordings of 5 or 10 minutes; see Baumert et al., 2009; El-Sheikh et al., 2011; Greaves-Lord et al., 2010). Therefore, the results from this study are highly valuable in terms of external validity and replicability. The findings supported how accurate nonlinear measures could be (in this instance, the allometric exponent *h*) in distinguishing between adolescents suffering from subclinical anxiety and controls. Conversely, a classical index of HRV, HF power, was not as sensitive as expected in characterizing adolescents with high anxious symptomatology. Finally, and as allometric exponent *h* indexes the basic properties of human systems (see Bornas, 2016, pp. 127-153; West, 2006, pp. 182-191), such as multistability and initial condition dependence, a stronger relationship between anxious symptomatology and sensitivity to punishment and this exponent was found in comparison with the HRV-related marker. In this sense, adolescents with high levels of anxiety showed lower cardiac complexity (lower fractal dimension) under everyday life conditions and this loss of complexity was proved to be linked with increases in anxious symptomatology and increasing sensitivity to punishment as an FT-related temperamental risk factor. This study also supports how relevant nonlinear measures are (in this case, the allometric exponent *h*, as an invariance domain measure) as accurate biomarkers, even for individuals along the continuum of anxiety but lying outside the region of a clinical diagnosis. Nevertheless,

linear cardiac biomarkers of anxiety may not be sensitive enough to identify individuals with subclinical anxiety.

With regard to Study 4, the aim was to explore the cardiac profile of at-risk individuals for ADs when faced with a stress induction paradigm (reactivity task). At-risk individuals were defined as adolescents who showed a rising trajectory of anxiety in a year. Taking into account the general trend of overall anxiety throughout middle adolescence (see Study 2), an increasing trajectory of anxiety may lead to an escalation of symptoms toward the development of an AD. As a result, two cardiac measures (HR and sample entropy) showed significantly lower levels in the at-risk participants when compared to their counterparts (adolescents with non-increasing trajectories) when undergoing the stress induction. These findings supported the hypothesis of sympathetic hypoactivation when stressful or challenging situations are faced (Chida & Hamer, 2008; Klumbies et al., 2014; Petrowski et al., 2017; Siess et al., 2013; Schmitz et al., 2013). Furthermore, it is worth noting that the stress induction paradigm as a laboratory-based, controlled protocol seemed useful in studying the distinctive properties of the cardiac system of adolescents at risk for ADs. Finally, two nonlinear biomarkers (DFA exponent α1, as an indicator of high anxious symptomatology, and sample entropy, as an indicator of a rising trajectory of symptoms) were proved to be sensitive biomarkers of subclinical maladaptive anxiety within this reactivity task.

The escalation of anxiety toward a disorder is a potential target for prevention programs in order to eliminate the noxious effects of clinical maladaptive anxiety in adolescents. Accordingly, Study 5 intended to test the efficacy of a prevention program the SUPER-Ad, in its school-based version, to prevent the escalation of anxiety in individuals with a 6-month rising trajectory of symptoms. Using the theoretical background on therapy outcomes by Bornas, Noguera, Pincus and Buela-Casal (2014), it assumed that a high intensity intervention (a multi-component intervention with some empirical support: the SUPER-Ad program) would modify a rising trajectory of symptoms but not a low intensity intervention (a school-work program). Results from this study supported this hypothesis, highlighting how valuable this transdiagnostic intervention was for preventing anxiety escalation. Moreover, these results are certainly

robust given that some experimental controls were put in place for this study (e.g., random allocation of conditions, a placebo and a waiting list group) and substantial effects were seen delivering the SUPER-Ad program, as demonstrated for transdiagnostic prevention programs (see Garcia-Escalera et al., 2016).

In short, this doctoral dissertation aimed at shedding light on the search for markers of maladaptive anxiety in adolescence and providing valuable tools for preventing the escalation of clinical anxiety. Flexibility may be a key concept for these purposes: first, a flexible, non-categorical approach in considering psychopathology (the RDoC initiative) has been adopted throughout the doctoral dissertation. This enables individuals with different conditions on the continuum of anxiety to be considered. Second, taking a flexible approach to analyzing subjective markers of anxiety (based on latent mixed modeling) has allowed an accurate picture on how anxiety symptomatology evolves over middle adolescence and how some relevant moderating factors (e.g., temperament and gender) may alter the developmental course of anxiety to be sketched. Third, (lack of) flexibility (or complexity) of the cardiac system should be deemed as a serious candidate in the search for biomarkers of anxiety in adolescence (see studies 3 and 4). Finally, the delivery of a flexible, transdiagnostic intervention (the SUPER-Ad program) proved to be effective in eliminating the rigid, inflexible trajectory of rising anxiety in middle adolescents.

By way of limitations, studies collected within this doctoral dissertation show some shortcomings that should be highlighted: first, studies compiled in this doctoral dissertation focused on early and middle adolescence. Late adolescence was therefore overlooked. Some studies highlight the importance of this period for the development of subsequent anxiety problems (Latvala et al., 2016; Wolitzky-Taylor et al., 2014). However, a concentration on specific periods in adolescence was taken in these studies in order to disentangle how anxiety evolves, as well as to determine the specific markers and risk factors in these periods. Second, a more integrative study of anxiety should have been developed, comprising other physiological systems (e.g., endocrine or digestive dynamics, among others) involved in anxiety manifestation (see Kreibig, 2010; Roth, 2005; Thayer & Sternberg, 2006). In this regard, studying heart dynamics

would be a cheap, non-invasive and easy-to-implement alternative for investigating anxiety. Moreover, the cardiac system is highly sensitive to context demands and its fluctuations can be easily detected with different devices (Allen et al., 2014; Cann et al., 1996). Finally, the studies included in this doctoral dissertation presented a gap in terms of risk factors. In other words, contextual risk factors for anxiety were rather disregarded in some of the studies. Nevertheless, a focus on stressful events (see Study 4) was considered as a way to look at how adolescents may react to these events in naturalistic contexts. Moreover, the impact of very stressful or traumatic events was also controlled in some of the studies (studies 3-5) as adolescents were undergoing a diagnostic interview to discard the presence of a PTSD (as well as other anxiety and OCD-related disorders).

Anxiety is a health hazard with an important impact on wellbeing and daily functioning. Studies in the current doctoral dissertation place a spotlight on integrating developmental and psychopathological findings so as to identify and ameliorate the noxious effects of anxiety during a crucial period in life, such as adolescence, by taking a flexible and groundbreaking approach.

3.1. Clinical Implications

Findings from the studies in this doctoral dissertation aim at encouraging a change in the traditional standpoint taken in clinical settings. As anxiety is a complex and multidimensional phenomenon, taking a flexible approach involves attaining knowledge to complement assessment and clinical intervention. Moreover, and due to the methodological controls taken in these studies, the feasibility of applying the subsequent conclusions to the real world is ensured.

In terms of clinical assessment, this doctoral dissertation highlights the influence of individual factors on the development of maladaptive anxiety. As a result, different types of anxiety trajectories may be found. This is paramount for selecting assessment

instruments and promoting the implementation of tailored protocols. Thus, a focus on temperamental factors taking a longitudinal perspective should be considered, given their enduring impact on anxiety. Adolescents are experiencing a maturation process and static assessments are strongly misleading, tending to represent unrealistic pictures of natural processes. In turn, psychological and clinical assessment must be supplemented with objective assessment. This doctoral dissertation proposes monitoring cardiac system function as a part of clinical assessment protocols due to its relatively low cost, easy implementation and absence of side effects. Of course, developmental aspects must be taken into consideration when studying the cardiac system (e.g., the relative parasympathetic dominance on heartbeat seen in adolescents and adults changes from early childhood), as stated in the Background section. Finally, the identification of nonlinear biomarkers of anxiety may provide some helpful hints for driving assessment protocols (e.g., the specific features that should be explored).

In terms of intervention, results from this doctoral dissertation encourage the delivery of tailored interventions. As a result of identifying different anxiety trajectories across adolescence, interventions need to be adapted to an individual's profile based on the risk and moderating factors for anxiety so as to make them more effective. Further, new intervention protocols may be derived from the findings observed in this thesis, especially in terms of physiological responses to contextual demands. For instance, new systems of biofeedback may be developed incorporating some nonlinear measures when monitoring cardiac activity.

In terms of prevention, preventive programs for anxiety should be encouraged and promoted by governments and decision-making institutions. Prevention constitutes a highly valuable option in many fields of health (e.g., drug use prevention, oncology, etc.), with substantial benefits in terms of reducing economic costs and promoting wellbeing. Unfortunately, and perhaps because of the difficulty in observing positive effects over the short-term, investment and research in preventing emotional problems is currently a pending issue. This dissertation sets out a valuable tool for anxiety prevention and its efficacy in reducing anxiety escalation was tested. The prevention program has been requested by several high school boards for ongoing delivery thanks

to its good results. Moreover, this preventive intervention may lead to the development of new preventive tools that are even more adapted to the requirements of specific populations. Prevention involves ensuring healthy maturation and good quality of life for adolescents.

3.2. Future Research Directions

As mentioned in previous chapters, this doctoral dissertation aimed to fill some important research gaps and overcome some shortcomings of studies in the field of anxiety in adolescence, taking a groundbreaking approach. As is always the case in science, research leads to new challenges that need to be addressed.

Would there be different classes of anxiety escalation depending on specific stressful contextual conditions (e.g., chronic stress conditions and experiencing traumatic events)? This question involves examining how anxiety escalates as a result of being immersed in certain contextual conditions, such as chronic stressful conditions or having been exposed to traumatic events. In this regard, shorter timescales of anxiety assessment would be useful in research protocols. Ecological momentary assessment (EMA) protocols would provide some valuable evidence in this vein with the added advantage of ecological data being collected (see Shiffman, Stone, & Hufford, 2008). EMA protocols have extensively been used for evaluating various psychopathological issues (Moore, Depp, Wetherell, & Lenze, 2016; Walz, Nauta, & aan het Rot, 2014). Combining these protocols with analytic strategies for identifying person-centered trajectories from an overall time-dependent trend (e.g., latent mixed modeling) enable the varying profiles of anxiety escalation under the influence of these contextual conditions to be characterized.

Another research question could be: *how can nonlinear biomarkers distinguish between individuals with clinical anxiety and non-clinical anxiety?* Addressing this issue involves characterizing the distinctive nature of full-blown disorders and several

subclinical conditions by means of nonlinear dynamics. Likewise, longitudinal or developmental aspects should be taken into consideration again. Some studies have postulated sensitive periods for different ADs throughout life (see Kessler et al., 2012; McDowell et al., 2014). Moreover, the way that anxiety impacts different functional domains also differs throughout life (see Copeland et al., 2014; Dotson et al., 2014).

Another important issue is related to physiological systems. The research question to be addressed here is: *how can nonlinear measures be researched for adolescents with anxiety taking into account other physiological systems?* As found in Study 1 in this doctoral dissertation, different nonlinear markers were found depending on the physiological system. For this reason, it would be relevant to study how other anxiety-related systems (e.g., the HPA system or brain electrophysiological system) function under the influence of adolescent anxiety. Searches in leading scientific databases fail to bring back any studies in the field of adolescent anxiety with any physiological system other than the heart. This fact underscores the current partial and incomplete picture of the complex phenomenon of anxiety in adolescence.

Finally, and more in line with therapeutics, the last research question is: *could nonlinear biomarkers be sensitive to therapeutic changes?* Some studies have pointed out that certain physiological responses may predict anxiety later in life (see Greaves-Lord et al., 2010). In the same vein, some studies have highlighted the changes observed in certain physiological systems as a result of delivering treatments for ameliorating anxiety (Bornas et al., 2012; Lueken et al., 2016). A complementary line of research could look into how preventive interventions that ease anxiety escalation lead to changes in the functioning of the physiological system and how these changes may predict the subsequent course of anxiety over time.

References 4

Adolph, D., & Margraf, J. (2017). The differential relationship between trait anxiety, depression, and resting frontal α-asymmetry. *Journal of Neural Transmission, 124,* 379-386.

Agorastos, A., Boel, J. A., Heppner, P. S., Hager, T., Moeller-Bertram, T., Haji, U., … Stiedl, O. (2013). Diminished vagal activity and blunted diurnal variation of heart rate dynamics in posttraumatic stress disorder. *Stress, 16*, 300-310.

Ahlen, J., Lenhard, F., Ghaderi, A. (2015). Universal prevention for anxiety and depressive symptoms in children: a meta-analysis of randomized and clusterrandomized trials. *Journal of Primary Prevention, 36*, 387-403.

Aldao, A., Nolen-Hoeksema, S., Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: a meta-analytic review. *Clinical Psychology Review, 30*, 217-237.

Alkozei, A., Creswell, C., Cooper, P. J., & Allen, J. J. B. (2015). Autonomic arousal in childhood anxiety disorders: associations with state anxiety and social anxiety disorder. *Journal of Affective Disorders, 175*, 25-33.

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience and Biobehavioral Reviews, 38*, 94-124.

Alvares, G. A., Quintana, D. S., Kemp, A. H., van Zwieten, A., Balleine, B. W., Hickie, I. B., & Guastella, A. J. (2013). Reduced heart rate variability in social anxiety disorder: associations with gender and symptom severity. *PLoS One, 8*, e70468.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: Author.

Anderson, P. L., Price, M., Edwards, S. M., Obasaju, M., Schmertz, S. K., Zimand, E., & Calamaras, M. R. (2013). Virtual reality exposure therapy for social anxiety disorder: a randomized controlled trial. *Journal of Consulting and Clinical Psychology, 81*, 751-760.

Asselmann, E., & Beesdo-Baum, K. (2015). Predictors of the course of anxiety disorders in adolescents and young adults. *Current Psychiatry Reports, 17*, 7.

Australian Psychological Society. (1997). *Guidelines for the use of psychological tests*. Sydney: Author.

Balasz, J., Miklosi, M., Kereszteny, A., Hoven, C. W., Carli, V., Wasserman, C., … Wasserman, D. (2013). Adolescent subthreshold-depression and anxiety: Psychopathology, functional impairment and increased suicide risk. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 54*, 670-677.

Balle, M., & Tortella-Feliu, M. (2010). Efficacy of a brief school-based program for selective prevention of childhood anxiety. *Anxiety, Stress, and Coping, 23*, 71-85.

Balle, M., Tortella-Feliu, M., & Bornas, X. (2013). Distinguishing youths at risk for anxiety disorders from self-reported BIS sensitivity and its psychophysiological concomitants. *International Journal of Psychology, 48*, 964-977.

Bandelow, B., & Michaelis, S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in Clinical Neuroscience, 17*, 327-335.

Bandelow, B., Baldwin, D., Abelli, M., Altamura, C., Dell'Osso, B., Domschke, K., ... Riederer, P. (2016). Biological markers for anxiety disorders, OCD and PTSD – a consensus statement. Part I: neuroimaging and genetics. *World Journal of Biological Psychiatry, 17*, 321-365.

Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Borin, M., Chamberlain, S. R., ... Riederer, P. (2017). Biological markers for anxiety disorders, OCD and PTSD: a consensus statement. Part II: neurochemistry, neurophysiology and neurocognition. *World Journal of Biological Psychiatry, 17*, 162-214.

Banihashemi, L., Sheu, L. K., Midei, A. J., & Gianaros, P. J. (2015). Childhood physical abuse predicts stressor-evoked activity within central visceral control regions. *Social Cognition and Affective Neuroscience, 10*, 474-485.

Barlow, D. H. (2002). *Anxiety and its disorders – The nature and treatment of anxiety and panic* (2nd ed.). New York, NY: The Guilford Press.

Barlow, D. H. (2004). Toward a unified treatment for emotional disorders. *Behavior Therapy, 35*, 205-230.

Bas-Hoogendam, J. M., Blackford, J. U., Bruhl, A. B., Blair, K. S., van der Wee, N. J. A., & Westenberg, P. M. (2016). Neurobiological candidate endophenotypes of social anxiety disorder. *Neuroscience and Biobehavioral Reviews, 71*, 362-378.

Baumert, M., Lambert, G. W., Dawood, T., Lambert, E. A., Esler, M. D., McGrane, M., & Nalivaiko, E. (2009). Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder. *American Journal of Physiology. Heart Circulation Physiology, 297*, H649-H674.

Baxter, A. J., Scott, K. M., Vos, T., & Whiteford, H. A. (2013). Global prevalence of anxiety disorders: a systematic review and meta-regression. *Psychological Medicine, 43*, 897-910.

Beauchaine, T. P., & Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. *International Journal of Psychophysiology, 98*, 338-350.

Beesdo-Baum, K., & Knappe, S. (2012). Developmental epidemiology of anxiety disorders. *Child and Adolescent Psychiatric Clinics of North America, 21*, 457-478.

Bentley, K. H., Gallagher, M. W., Boswell, J. F., Gorman, J. M., Shear, M. K., Woods, S. W., & Barlow, D. H. (2013). The interactive contributions of perceived control and anxiety sensitivity in panic disorder: a triple vulnerabilities perspective. *Journal of Psychopathology and Behavioral Assessment, 35*, 57-64.

Berk, M., Ng, F., Wang, W., Calabrese, J., Mitchell, P., Malhi, G., & Tohen, M. (2008). The empirical redefinition of the psychometric criteria for remission in bipolar disorder. *Journal of Affective Disorders, 106,* 153-158.

Berking, M., & Whitley, B. (2014). Emotion regulation: definition and relevance for mental health. In *Affect regulation training: a practitioners' manual* (doi: 10.1007/978-1-4939-1022-9_2). New York (NY): Springer Science + Business Media.

Betts, K. S., Bakker, P., Alati, R., McIntosh, J. E., MacDonald, J. A., Letcher, P., & Olsson, C. A. (2016). The natural history of internalizing behaviours from adolescence to emerging

adulthood: findings from the Australian Temperament Project. Psychological Medicine, 46, 2815-2827.

Bijttebier, P., Beck, I., Claes, L., & Vandereycken, W. (2009). Gray's Reinforcement Sensitivity Theory as a framework for research on personality-psychopathology associations. *Clinical Psychology Review, 29,* 421-430.

Bilek, E. L., & Ehrenreich-May, J. (2012). An open trial investigation of a transdiagnostic group treatment for children with anxiety and depressive symptoms. *Behavior Therapy, 43,* 887-897.

Blair, K. S., Geraci, M., Smith, B. W., Hollon, N., DeVido, J., Otero, M., ... Pine, D. S. (2012). Reduced dorsal anterior cingulate cortical activity during emotional regulation and top-down attentional control in generalized social phobia, generalized anxiety disorder, and comorbid generalized social phobia/generalized anxiety disorder. *Biological Psychiatry, 72,* 476-482.

Blom, E. H., Olsson, E. M., Serlachius, E., Ericson, M., & Ingvar, M. (2010). Heart rate variability (HRV) in adolescent females with anxiety disorders and major depressive disorder. *Acta Paediatrica, 99,* 604-611.

Bluett, E. J., Homan, K. J., Morrison, K. L., Levin, M. E., & Twohig, M. P. (2014). Acceptance and commitment therapy for anxiety and OCD spectrum disorders: an empirical review. *Journal of Anxiety Disorders, 28,* 612-624.

Bongers, I. L., Koot, H. M., van der Ende, J., & Verhulst, F. C. (2003). The normative development of child and adolescent problem behavior. *Journal of Abnormal Psychology, 112,* 179-192.

Bornas, X. (2016). *Psicopatología y caos* [Psychopathology and chaos] (2a ed.). Madrid: Bubok.

Bornas, X., Balle, M., de la Torre-Luque, A., Fiol-Veny, A., & Llabres, J. (2015). Ecological assessment of heart rate complexity: differences between high- and low-anxious adolescents. *International Journal of Psychophysiology, 98,* 112-118.

Bornas, X., Llabres, J., Balle, M., de la Torre-Luque, A., & Fiol-Veny, A. (2014). The use of a secure social network for the study of anxiety in adolescence. *Revista de Psicopatología y Psicología Clínica, 19*, 171-184.

Bornas, X., Llabres, J., Noguera, M., Lopez, A. M., Gelabert, J. M., & Vila, I. (2006). Fear induced complexity loss in the electrocardiogram of flight phobics: a multiscale entropy analysis. *Biological Psychology, 73*, 272-279.

Bornas, X., Noguera, M., Pincus D., & Buela-Casal, G. (2014). Emotional inertia: a key to understanding psychotherapy process and outcome. *International Journal of Clinical and Health Psychology, 14*, 232-239.

Bornas, X., Riera, A., Tortella-Feliu, M., & Llabres, J. (2012). Heart rate variability profiles and exposure therapy treatment outcome in flight phobia. *Applied Psychophysiology and Biofeedback, 37*, 53-62.

Bosquet, M., & Egeland, B. (2006). The development and maintenance of anxiety symptoms from infancy through adolescence in a longitudinal sample. *Development and Psychopathology, 18*, 517-550.

Bouwer, C., & Stein, D. J. (1998). Use of the selective serotonin reuptake inhibitor citalopram in the treatment of generalized social phobia. *Journal of Affective Disorders, 49*, 79-82.

British Psychological Society. (2017). *Psychological testing: a user's guide.* Retrieved from http://cmapspublic3.ihmc.us/rid=1M4F4519G-P2JSFT-4KM/Psychological%20Testing%20%20A%20Users%20Guide.pdf

Brook, C. A., & Schmidt, L. A. (2008). Social anxiety disorder: a review of environmental risk factors. *Neuropsychiatric Disease and Treatment, 4*, 123-143.

Brosschot, J. F. (2010). Markers of chronic stress: prolonged physiological activation and (un)conscious perseverative cognition. *Neuroscience and Biobehavioral Reviews, 35*, 46-50.

Burstein, M., Beesdo-Baum, K., He, J.-P., & Merikangas, K. R. (2014). Threshold and subthreshold generalized anxiety disorder among US adolescents: prevalence, sociodemographic, and clinical characteristics. *Psychological Medicine, 44*, 2351-2362.

Buschges, A. (2005). Sensory control and organization of neural networks mediating coordination of multisegmental organs for locomotion. *Journal of Neurophysiology, 93*, 1127-1135.

Caldirola, D., Bellodi, L., Caumo, A., Migliarese, G., Perna, G., 2004. Approximate entropy of respiratory patterns in panic disorder. *American Journal of Psychiatry, 161*, 79-87.

Camm, A. J., Malik, M., Bigger, J. T.,Breithardt, G., Cerutti, S., Cohen, R. J., ... Singer, D. H. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17*, 354-381.

Capaldi, D. M., & Rothbart, M. K. (1992). Development and validation of an early adolescent temperament measure. *Journal of Early Adolescence, 12*, 153-173.

Carmassi, C., Gesi, C., Massimetti, E., Shear, M. K., & Dell'Osso, L. (2015). Separation anxiety disorder in the DSM-5 era. *Journal of Psychopathology, 21*, 365-371.

Carroll, B. J., Iranmanesh, A., Keenan, D. M., Cassidy, F., Wilson, W. H., & Veldhuis, J. D. (2012). Pathophysiology of hypercortisolism in depression: pituitary and adrenal responses to low glucocorticoid feedback. *Acta Psychiatrica Scandinavica, 125*, 478-491.

Casey, B. J., Oliveri, M. E., & Insel, T. R. (2014). A neurodevelopmental oerspective on the Research Domain Criteria (RDoC) framework. *Biological Psychiatry, 76*, 350-353.

Caspi, A., Houts, R. M., Belsky, D. W., Goldman-Mellor, S. J., Harrington, H., Israel, S., ... Moffitt, T. E. (2014). The p factor: one general psychopathology factor in the structure of psychiatric disorders? *Clinical Psychological Science, 2*, 119-137.

Chae, J.-H., Jeong, J., Peterson, B. S., Kim, D.-J., Bahk, W.-M., Jun, T.-Y., ... Kim, K. S. (2004). Dimensional complexity of the EEG in patients with posttraumatic stress disorder. *Psychiatry Research, 131*, 79-89.

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., & Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Frontiers in Psychiatry, 5*, 80.

Chan, T. C., Sharieff, G. Q., & Brady, W. J. (2008). Electrocardiographic manifestations: pediatric ECG. *Journal of EmergencyMedicine, 35*, 421-430.

Chialvo, D. R. (2010). *Emergent complex neural dynamics*. Nature Physics, 6, 744-750.

Chida, Y., & Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. *Psychological Bulletin, 134*, 829-885.

Chorpita, B. F., Moffit, C. E., & Gray J. (2005). Psychometric properties of the Revised Child Anxiety and Depression Scale in a clinical sample. *Behaviour Research and Therapy, 43*, 309-322.

Cicchetti, D. (1993). Developmental psychopathology: reactions, reflections, projections. *Developmental Review, 13*, 471-502.

Cisler, J. M., & Olatunji, B. O. (2012). Emotion regulation and anxiety disorders. *Current Psychiatry Reports, 14*, 182-187.

Cisler, J. M., Olatunji, B. O., Feldner, M. T., & Forsyth, J. P. (2010). Emotion regulation and the anxiety disorders: an integrative review. *Journal of Psychopathology and Behavioral Assessment, 32*, 68-82.

Clark, C. R., Galletly, C. A., Ash, D. J., Moores, K. A., Penrose, R. A., & McFarlane, A. C. (2009). Evidence-based medicine evaluation of electrophysiological studies of the anxiety disorders. *Clinical EEG and Neuroscience, 40*, 84-112.

Clark, D. A. (2009). Cognitive behavioral therapy for anxiety and depression: possibilities and limitations of a transdiagnostic perspective. *Cognitive Behaviour Therapy, 38*, 29-34.

Clement, Y., Calatayud, F., & Belzung, C. (2002). Genetic basis of the anxiety-like behaviour: a critical review. *Brain Research Bulletin, 57*, 57-71.

Conrad, A., & Roth, W. T. (2007). Muscle relaxation therapy for anxiety disorders: it works but how? *Journal of Anxiety Disorders, 21*, 243-264.

Copeland, W. E., Angold, A., Shanahan, L., & Costello, J. (2014). Longitudinal patterns of anxiety from childhood to adulthood: the Great Smoky Mountains Study. *Journal of the American Academy of Child and Adolescent Psychiatry, 53*, 21-33.

Costello, E. J., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry, 60,* 837-844.

Craske, M. G. (2012). Transdiagnostic treatment for anxiety and depression. *Depression and Anxiety, 29,* 749-753.

Craske, M. G., Rauch, S. L., Ursano, R., Preonoveau, J., Pine, D. S., & Zinbarg, R. E. (2009). What is an anxiety disorder? *Depression and Anxiety, 26,* 1066-1085.

Croarkin, P. E., Levinson, A. J., & Daskalakis, Z. J. (2011). Evidence for GABAergic inhibitory deficits in major depressive disorder. *Neuroscience and Biobehavioral Reviews, 35,* 818-825.

Damsa, C., Kosel, M., & Moussally, J. (2009). Current status of brain imaging in anxiety disorders. *Current Opinion in Psychiatry, 22,* 96-110.

Dantan, E., Proust-Lima, C., Letenneur, L., & Jacqmin-Gadda, H. (2008). Pattern mixture models and latent class models for the analysis of multivariate longitudinal data with informative dropouts. *International Journal of Biostatistics, 4,* 14.

Dark, T., Flynn, H. A., Rust, G., Kinsell, H., & Harman, J. S. (2016). Epidemiology of emergency department visits for anxiety in the United States: 2009-2011. *Psychiatric Services in Advance, 68,* 238-244.

Davey, H. M., Barratt, A. L., Butow, P. N., & Deeks, J. J. (2007). A one-item question with a likert or visual analog scale adequately measured current anxiety. *Journal of Clinical Epidemiology, 60,* 356-360.

De Carvalho, H. W., Andreoli, S. B., Lara, D. R., Patrick, C. J., Quintana, M. I., Bressan, R. A., ... Jorge, M. R. (2014). The joint structure of major depression, anxiety disorders, and trait negative affect. *Revista Brasileira de Psiquiatria, 36,* 285-292.

De la Torre-Luque, A., Balle, M., Fiol-Veny, A., Llabres, J., & Bornas, X. (under review). Sustained anxiety scores are related with the fractal dynamics of the heartbeat in early adolescents. *Nonlinear Dynamics, Psychology, and Life Sciences.*

De la Torre-Luque, A., Diaz-Piedra, C., & Buela-Casal, G. (2017). Effects of preferred relaxing music after acute stress exposure: a randomized controlled trial. *Psychology of Music*. doi: 10.1177/0305735617689953

De la Torre-Luque, A., Essau, C. A., Fiol-Veny, A., Balle, M., & Bornas, X. (2015). *Super Skills for Life for Spanish adolescents: the SUPER-Ad programme*. Poster presented at European Symposium on Adolescent Research & Research Method Workshop (London, December, 2015).

De Pauw, S. S. W., & Mervielde, I. (2010). Temperament, personality and developmental psychopathology: A review based on the conceptual dimensions underlying childhood traits. *Child Psychiatry and Human Development, 41*, 313-329.

Dieleman, G. C., Huizink, A. C., Tulen, J. H. M., Utens, W. M. W. J., Creemers, H. E., van der Ende, J., & Verhulst, F. C. (2015). Alterations in HPA-axis and autonòmic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis. *Psychoneuroendocrinology, 51*, 135-150.

Domschke, K., & Reif, A. (2011). Behavioral genetics of affective and anxiety disorders. *Current Topics in Behavioral Neuroscience, 12*, 463-502.

Dotson, V. M., Szymkowicz, S. M., Kirton, J. W., McLaren, M. E., Green, M. L., & Rohani, J. Y. (2014). Unique and interactive effect of anxiety and depressive symptoms on cognitive and brain function in young and older adults. *Journal of Depression and Anxiety, Suppl 1*, 22565.

Dozois, D. J. A., Seeds, P. M., & Collins, K. A. (2009). Transdiagnostic approaches to the prevention of depression and anxiety. *Journal of Cognitive Psychotherapy, 23*, 44-59.

Drake, K. L., & Ginsburg, G. S. (2012). Family factors in the development, treatment, and prevention of childhood anxiety disorders. *Clinical Child and Family Psychology Review, 15*, 144-162.

Duncko, R., Makatsori, A., Fickova, E., Selko, D., & Jezova, D. (2006). Altered coordination of the neuroendocrine response during psychosocial stress in subjects with high trait anxiety. *Progress in Neuro-psychopharmacology and Biological Psychiatry, 30*, 1058-1066.

Duval, E. R., Javanbakht, A., & Liberzon, I. (2015). Neural circuits in anxiety and stress disorders: a focused review. *Therapeutics and Clinical Risk Management, 11*, 115-126.

Eiland, L., & Romeo, D. (2013). Stress and the developing adolescent brain. *Neuroscience, 249*, 162-171.

Ellis, J. L. (2013). A standard for test reliability in group research. *Behavior Research Methods, 45*, 16-24.

Elosua, P. (2003). About test validity. *Psicothema, 15*, 515-521.

El-Sheikh, M., & Arsiwalla, D. D., Hinnant, J. B., & Erath, S. A. (2011). Children's internalizing symptoms: the role of interactions between cortisol and respiratory sinus arrhythmia. *Physiology and Behavior, 103*, 225-232.

Erhardt, A., Czibere, L., Roeske, D., Lucae, S., Unschuld, P. G., Ripke, S., ... Binder, E. B. (2011). TMEM132D, a new candidate for anxiety phenotypes: evidence from human and mouse studies. *Molecular Psychiatry, 16*, 647-663.

Essau, C. A., & Gabbidon, J. (2013). Epidemiology, comorbidity and mental health services utilization. In C. A. Essau and T. H. Ollendick (Eds.), *The Wiley-Blackwell handbook of the treatment of childhood and adolescent anxiety* (pp. 23-42). Malden, MA: John Wiley & Sons, Ltd.

Essau, C. A., & Ollendick, T. H. (2013). *The Super Skills for Life programme.* London: University of Roehampton.

Essau, C. A., Lewinsohn, P. M., Olaya, B., & Seeley, J. R. (2014). Anxiety disorders in adolescents and psychosocial outcomes at age 30. *Journal of Affective Disorders, 163*, 125-132.

Etkin, A., & Cuthbert, B. (2014). Beyond the DSM: development of a transdiagnostic psychiatric neuroscience course. *Academic Psychiatry, 38*, 145-150.

Eyre, E. L. J., Duncan, M. J., Birch, S. L., & Fisher, J. P. (2014). The influence of age and weight status on cardiac autonomic control in healthy children: a review. *Autonomic Neuroscience: Basic and Clinical, 186*, 8-21.

Faravelli, C., Lo Sauro, C., Lelli, L., Pietrini, F., Lazzaretti, L., Godini, L., ... Ricca, V. (2012). The role of life events and HPA axis in anxiety disorders: a review, *Current Pharmaceutical Design*, *18*, 5663-5674.

Farchione, T. J., Fairholme, C. P., Ellard, K. K., Boisseau, C. L., Thompson-Hollands, J., Carl, J. R., Gallagher, M. W., & Barlow, D. H. (2012). Unified protocol for transdiagnostic treatment of emotional disorders: a randomized controlled trial. *Behavior Therapy, 43*, 666-678.

Fehm, L., Beesdo-Baum, K., Jacobi, F., Fiedler, A. (2008). Social anxiety disorder above and below the diagnòstic threshold: prevalence, comorbidity and impairment in the general population. *Social Psychiatry and Psychiatric Epidemiology, 43*, 257-265.

Ferdinand, R. F., Dieleman, G., Ormel, J., & Verhulst, F. C. (2007). Homotypic versus heterotypic continuity of anxiety symptoms in young adolescents: evidence for distinctions between DSM-IV subtypes. *Journal of Abnormal Child Psychology, 35*, 325-333.

Ferro, M. A., & Boyle, M. H. (2015). The impact of chronic physical illness, maternal depressive symptoms, family functioning, and self-esteem on symptoms of anxiety and depression in children. *Journal of Abnormal Child Psychology, 43*, 177-187.

Fiol-Veny, A., de la Torre-Luque, A., Balle, M., & Bornas, X. (2017). Allometric control of affective fluctuations in early adolescents: their association with anxiety symptoms and temperament traits. *Nonlinear Dynamics, Psychology, and Life Sciences, 21*, 255-266.

Fisher, A. J., & Newman, M. G. (2013). Heart rate and autonomic response to stress after experimental induction of worry versus relaxation in healthy, high-worry, and generalized anxiety disorder individuals. *Biological Psychology, 93*, 65-74.

Fleming, S., Thompson, M., Stevens, R., Heneghan, C., Pluddemann, I., Tarassenko, L., & Mant, D. (2011). Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. *Lancet, 377*, 1011-1018.

Francati, V., Vermetten, E., & Bremner, J. D. (2007). Functional neuroimaging studies in posttraumatic stress disorder: review of current methods and findings. *Depression and Anxiety, 24*, 202-218.

Freeman, W. J. (1999). *How brains make up their minds*. London: Weidenfeld and Nicolson.

Frick, P. J., Barry, C. T., & Kamphaus, R. W. (2010). *Clinical assessment of child and adolescent personality and behavior* (3rd ed). New York, NY: Springer Science + Business Media.

Friedman, B. H. (2007). An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. *Biological Psychology, 74*, 185-199.

Friedman, M. J., Resick, P. A., Bryant, R. A., & Brewin, C. R. (2011). Considering PTSD for DSM-5. *Depression and Anxiety, 28*, 750-769.

Friedman, M. J., Resick, P. A., Bryant, R. A., Strain, J., Horowitz, M., & Spiegel, D. (2011). Classification of trauma and stressor-related disorders in DSM-5. *Depression and Anxiety, 28*, 737-749.

Furmark, T. (2009). Neurobiological aspects of social anxiety disorder. *Israel Journal of Psychiatry and Related Sciences, 46*, 5-12.

Gabella, G. (2001). Autonomous nervous System. In *Encyclopedia of Life Sciences* (doi: 10.1002/9780470015902.a0000081.pub2). John Wiley & Sons, Ltd.

Gaebler, M., Daniels, J. K., Lamke, J.-P., Fydrich, T., & Walter, H. (2013). Heart rate variability and its neural correlates during emotional face processing in social anxiety disorder. *Biological Psychology, 94*, 319-330.

Garcia-Escalera, J., Chorot, P., Valiente, R. M., Reales, J. M., & Sandin, B. (2016). Effcacy of transdiagnostic cognitivebehavioral therapy for anxiety and depression in adults, children and adolescents: a metaanalysis. *Revista de Psicopatologia y Psicologia Clinica, 21*, 147-175.

Garvey, M., Avenevoli, S., & Anderson, K. (2016). The National Institute of Mental Health Research Domain Criteria and clinical research in child and adolescent psychiatry. *Journal of the American Academy of Child & Adolescent Psychiatry, 55*, 93-98.

Gatt, J. M., Burton, K. L. O., Williams, L. M., & Schofield, P. R. (2015). Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. *Journal of Psychiatric Research, 60*, 1-13.

Gillham, J. E., Reivich, K. J., Freres, D. R., Lascher, M., Litzinger, S., Shatté, A., & Seligman, M. E. P. (2006). School-based prevention of depression and anxiety symptoms in early adolescence: a pilot of a parent intervention component. *School Psychology Quarterly, 21*, 323-348.

Gleick, J. (1987). *Chaos: making a new science*. New York, NY: Viking Penguin.

Glover, V. (2011). Annual Research Review: prenatal stress and the origins of psychopathology: an evolutionary perspective. *Journal of Child Psychology and Psychiatry, 52*, 356-367.

Goddard, A. W. (2016). Cortical and subcortical gamma amino acid butyric acid deficits in anxiety and stress disorders: clinical implications. *World Journal of Psychiatry, 22*, 43-53.

Goldberger, A. L., Amaral, L. A., Hausdorff, J. M., Ivanov, P. C., Peng, C. K., & Stanley, H.E. (2002). Fractal dynamics in physiology: alterations with disease and aging. *Proceedings of the National Academy of Sciences of the United States of America, 19*, 2466-2472.

Gotham, K., Brunwasser, S. M., & Lord, C. (2015). Depressive and anxiety symptom trajectories from school age through young adulthood in samples with autism spectrum disorder and developmental delay. *Journal of the American Academy of Child and Adolescent Psychiatry, 54*, 369-376.

Graber, J. A., Brooks-Gunn, J., & Peterson, A. C. (1996). *Transitions through adolescence: Interpersonal domains and context*. Mahwah, NJ: Lawrence Erlbaum Associates.

Graeff, F. G., & Zangrossi, H., Jr. (2010). The hypothalamic-pituitary-adrenal axis in anxiety and pànic. *Psychology and Neuroscience, 3*, 3-8.

Granic, I. (2005). Timing is everything: developmental psychopathology from a dynamic systems perspective. *Developmental Review, 25*, 386-407.

Grant, B. F., Goldstein, R. B., Chou, S. P., Huang, B., Stinson, F. S., Dawson, D. A., ... Compton, W. M. (2009). Sociodemographic and psychopathologic predictors of first incidence of DSM-IV substance use, mood and anxiety disorders: results from the Wave 2 National Epidemiologic Survey on Alcohol and Related Conditions. *Molecular Psychiatry, 14*, 1051-1066.

Gray, J. A. (1982). *The neuropsychology of anxiety: An enquiry into the functions of the septohippocampal system*. New York, NY: Clarendon Press/Oxford University Press.

Gray, J. A., & McNaughton, N. (2000). *The neuropsychology of anxiety*. Oxford: Oxford University Press.

Graziano, P., & Derefinko, K. (2013). Cardiac vagal control and children's adaptive functioning: a meta-analysis. *Biological Psychology, 94*, 22-37.

Greaves-Lord, K., Ferdinand, R. F., Oldehinkel, A. J., Sondeijker, F. E. P. L., Ormel, J., & Verhulst, F. C. (2007). Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta Psychiatrica Scandinavica, 116*, 137-144.

Greaves-Lord, K., Tulen, J., Dietrich, A., Sondeijker, F., van Roon, A., Oldehinkel, A., ... Huizink, A. (2010). *Psychiatry Research, 179*, 187-193.

Grenier, J., Greenman, P. S., Farmanova, E., Atchessi, N., Annous, R., & Chomienne, M. H. (2015). Scoping review of risk factors of anxiety disorders and post-traumatic stress disorder: a public health perspective. *Annals of Depression and Anxiety, 2*, 1068.

Gulewitsch, M. D., Muller, J., Enck, P., Weimer, K., Schwille-Kiuntke, J., & Schlarb, A. A. (2014). Frequent abdominal pain in childhood and youth: a systematic review of psychophysiological characteristics. *Gastroenterology Research and Practice, 2014*, 524383.

Gulley, L. D., Hankin, B. L., & Young, J. F. (2016). Risk for depression and anxiety in youth: the interaction between negative affectivity, effortful control, and stressors. *Journal of Abnormal Child Psychology, 44*, 207-218.

Gunnar, M. R., Wewerka, S., Frenn, K., Long, J. D., & Griggs, C. (2009). Developmental changes in hypothalamus–pituitary–adrenal activity over the transition to adolescence: normative changes and associations with puberty. *Developmental Psychopathology, 21*, 69-85.

Gustafsson, P. E., Gustafsson, P. A., Ivarsson, T., & Nelson, N. (2008). Diurnal cortisol levels and cortisol response in youths with obsessive-compulsive disorder. *Neuropsychobiology, 57*, 14-21.

Hall, G. S. (1904). *Adolescence: its psychology and its relations to physiology, anthropology, sociology, sex, crime, religion, and education*. New York, NY: Appleton.

Haller, H., Cramer, H., Lauche, R., Gass, F., & Dobos, G. J. (2014). The prevalence and burden of subthreshold generalized anxiety disorder: a systematic review. *BMC Psychiatry, 14*, 128.

Hampel, P., & Petermann, F. (2006). Perceived stress, coping, and adjustment in adolescents. *Journal of Adolescent Health, 38*, 409-415.

Harvey, A. G., Clark, D. M., Ehlers, A., & Rapee, R. M. (2000). Social anxiety and self-impression: Cognitive preparation enhances the beneficial effects of video feedback following a stressful social task. *Behaviour, Research and Therapy, 38*, 1183-1192.

Hashoul-Andary, R., Assayag-Nitzan, Y., Yuval, K., Aderka, I. M., Litz, B., & Bernstein, A. (2016). A longitudinal study of emotional distress intolerance and psychopathology following exposure to a potentially traumatic event in a community sample. *Cognitive Therapy and Research, 40*, 1-13.

Hauschildt, M., Peters, M. J. V., Moritz, S., & Jelinek, L. (2011). Heart rate variability in response to affective scenes in posttraumatic stress disorder. *Biological Psychology, 88*, 215-222.

Hausdorff, J. M., Mitchell, S. L., Firtion, R., Peng, C.-K., Cudkowicz, M. E., Wei, J. Y., & Goldberger, A. L. (1997). *Journal of Applied Physiology, 82*, 262-269.

Henderson, L., Gilbert, P., & Zimbardo, P. (2014). Syness, social anxiety, and social phobia. In S. G. Hofmann and P. M. DiBartolo (Eds.), *Social anxiety* (3rd ed, pp. 95-114). New York, NY: Elsevier, Inc.

Hirshfeld-Becker, D. R., Micco, J. A., Simoes, N. A., & Henin, A. (2008). High risk studies and developmental antecedents of anxiety disorders. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics, 148C*, 99-117.

Ho, K. K. L., Moody, G. B., Peng, C.-K., Mietus, J. E., Larson, M. G., Levy, D., & Goldberger, A. L. (1997). Predicting survival in heart failure case and control subjects by use of fully automated methods for derivin nonlinear and conventional indices of heart rate dynamics. *Circulation, 96*, 842-848.

Hollander, E., Kim, S., Braun, A., Simeon, D., & Zohar, J. (2009). Cross-cutting issues and future directions for the OCD spectrum. *Psychiatry Research, 170*, 3-6.

Hollenstein, T., & Lougheed, J. P. (2013). Beyond storm and stress: typicality, transactions, timing, and temperament to account for adolescent change. *The American Psychologist, 68*, 444-454.

Hollenstein, T., Lichtwarck-Aschoff, A., & Potworowski, G. (2013). A model of socioemotional flexibility at three time scales. *Emotion Review, 0*, 1-9.

Holzel, B. K., Hoge, E. A., Greve, D. N., Gard, T., Creswell, J. D., Brown, K. W., ... Lazar, S. W. (2013). Neural mechanisms of symptom improvements in generalized anxiety disorder following mindfulness training. *Neuroimage: Clinincal, 2*, 448-458.

Hornblow, A. R., & Kidson, M. A. (1976). The visual analogue scale for anxiety: a validation study. *Australian and New Zealand Journal of Psychiatry, 10*, 339-341.

Huizink, A. C. (2012). Prenatal substance use, prenatal stress and offspring behavioural outcomes: considerations for future studies. *Nordic Journal of Psychiatry, 66*, 115-122.

Insel, T. R. (2014). The NIMH Research Domain Criteria (RDoC) project: precision medicine for psychiatry. *American Journal of Psychiatry, 171*, 395-397.

Insel, T. R., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., ... Wang, P. (2010). Research Domain Criteria (RDoC): toward a new classifcation framework for research on mental disorders. *American Journal of Psychiatry, 167*, 748-751.

International Test Commission. (2001). ITC guidelines on test use. *International Journal of Testing, 1*, 93-114.

Ip, P., Wong, R. S., Li, S. L., Chan, K. L., Ho, F. K., & Chow, C.-B. (2016). Mental health consequences muellerof childhood physical abuse in chinese populations: a meta-analysis. *Trauma, Violence, and Abuse, 17*, 571-584.

Ivanov, P. C., Chen, Z., Hu, K., & Stanley, H. E. (2004). Multiscale aspects of cardiac control. *Physica A, 344*, 685-704.

Kamarova, D., Hajda, M., Prasko, J., Taborsky, J., Grambal, A., Latalova, K., ... Hlustik, P. (2016). Cognitive deficits in patients with obsessive-compulsive disorder –

electroencephalography correlates. *Neuropsychiatric Disease and Treatment, 12*, 1119-1125.

Karemaker, J. M. (2017). An introduction into autonòmic nervous function. *Physiological Measurement, 38*, R89.

Karevold, E., Ystrom, E., Coplan, R. J., Sanson, A. V., & Mathiesen, K. S. (2012). A prospective longitudinal study of shyness from infancy to adolescence: Stability, age-related changes, and prediction of socio-emotional functioning. *Journal of Abnormal Child Psychology, 40*, 1167-1177.

Karsten, J., Hartman, C. A., Smit, J. H., Zitman, F. G., Beekman, T. F., Cuijpers, P., ... Penninx, B. W. J. H. (2011). Psychiatric history and subthreshold symptoms as predictors of the occurrence of depressive or anxiety disorder within 2 years. *British Journal of Psychiatry, 198*, 206-212.

Katerndahl, D. A. (2009). Lessons from Jurassic Park: patients as complex adaptive. *Journal of Evaluation in Clinical Practice, 15*, 755-760.

Kenny, D. T., Fortune, J. M., & Ackermann, B. (2011). Predictors of music performance anxiety during skilled performance in tertiary flute players. *Psychology of Music, 41*, 306-328.

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A., & Wittchen, H.-U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *International Journal of Methods in Psychiatric Research, 21*, 169-184.

Kimmel, R. J., Roy-Byrne, P. P., & Cowley, D. S. (2015). Pharmacological treatments for panic disorder, generalized anxiety disorder, specific phobia, and social anxiety disorder. In P. E. Nathan and J. M. Gorman (Eds.), *A guide to treatments that work* (4th ed., pp. 463-506). New York, NY: Oxford University Press.

Kindt, K. C. M., Kleinjan, M., Janssens, J. M. A. M., & Scholte, R. H. J. (2014). Evaluation of a school-based depression prevention program among adolescents from low-income areas: a randomized controlled effectiveness trial. *International Journal of Environmental Research and Public Health, 11*, 5273-5293.

King, R. D., Brown, B., Hwang, M., Jeon, T., George, A. T., & Alzheimer's Disease Neuroimaging Initiative. (2010). Fractal dimension analysis of the cortical ribbon in mild Alzheimer's disease. *NeuroImage, 53*, 471-479.

King, S., Dancause, K., Turcotte-Tremblay, A.-M., Veru, F., & Laplante, D. P. (2012). Using natural disasters to study the effects of prenatal maternal stress on child health and development. *Birth Defects Research (Part C), 96*, 273-288.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The "Trier Social Stress Test": A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*, 76-81.

Klumbies, E., Braeuer, D., Hoyer, J., & Kirschbaum, C. (2014). The reaction to social stress in social phobia: discordance between physiological and subjective parameters. *PLoS One, 9*, e105670.

Knappe, S., Beesdo-Baum, K., Wittchen, H.-U. (2010). Familial risk factors in social anxiety disorder: calling for a family-oriented approach for targeted prevention and early intervention. *European Child and Adolescent Psychiatry, 19*, 857-871.

Knyazev, G. G. (2012). EEG delta oscillations as a correlate of basic homeostatic and motivational processes. *Neuroscience and Biobehavioral Reviews, 36*, 677-695.

Konnopka, A., Leichsenring, F., Leibing, E., & Konig, H.-H. (2009). Cost-of-illness studies and cost-effectiveness analyses in anxiety disorders: a systematic review. *Journal of Affective Disorders, 114*, 14-31.

Konorski, J. (1967). Some new ideas concerning the physiological mechanisms of perception. *Acta Biologiae Experimentalis, 27*, 147-161.

Koprivova, J., Congedo, M., Horacek, J., Prasko, J., Raszka, M., Brunovsky, M., … Hoschl, C. (2011). EEG source analysis in obsessive-compulsive disorder. *Clinical Neurophysiology, 122*, 1735-1743.

Kotov, R., Gamez, W., Schmidt, F., & Watson, D. (2010). Linking "Big" Personality Traits to anxiety, depressive, and substance use disorders: a meta-analysis. *Psychological Bulletin, 136*, 768-821.

Kowalewski, M., Alifier, M., Bochen, D., & Urban, M. (2007). Heart rate turbulence in children — age and heart rate relationships. *Pediatric Research, 62*, 710-714.

Kramer, M., Seefeldt, W. L., Heinrichs, N., Tuschen-Caffer, B., Schmitz, J., Wolf, O. T., & Blechert, J. (2012). Subjective, autonomic, and endocrine reactivity during social stress in children with social phobia. *Journal of Abnormal Child Psychology, 40*, 95-104.

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: a review. *Biological Psychology, 84*, 394-421.

Kyrou, I., & Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Current Opinion in Pharmacology, 9*, 787-793.

Laceulle, O. M., Nederhof, E., Karreman, A., Ormel, J., & van Aken, M. A. G. (2012). Stressful events and temperament change during early and middle adolescence: the TRAILS Study. *European Journal of Personality, 26*, 276-284.

Lang, P. J. (2010). Emotion and motivation: toward consensus definitions and a common research purpose. *Emotion Review, 2*, 229-233.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. *Biological Psychiatry, 44*, 1248-1263.

Latvala, A., Kuja-Halkola, R., Ruck, C., D'Onofrio, B. M., Jernberg, T., Almqvist, C., … Lichtenstein, P. (2016). Association of resting heart rate and blood pressure in late adolescence with subsequent mental disorders: a longitudinal population study of more than 1 million men in Sweden. *JAMA Psychiatry, 73*, 1268-1275.

Leadbeater, B., Thompson, K., & Gruppuso, V. (2012). Co-occurring trajectories of symptoms of anxiety, depression, and oppositional defiance from adolescence to young adulthood. *Journal of Clinical Child and Adolescent Psychology, 41*, 719-730.

Lee, K.-C., Chao, Y.-H., Yiin, J.-J., Chiang, P.-Y., & Chao, Y.-F. (2011). Effectiveness of different music-playing devices for reducing preoperative anxiety: a clinical control study. *International Journal of Nursing Studies, 48*, 1180-1187.

Leibold, N. K., van den Hove, D. L. A., Viechtbauer, W., Buchanan, G. F., Goossens, L., Lange, I., … Schruers, K. R. J. (2016). *Translational Psychiatry, 6*, e885.

Lindert, J., von Ehrenstein, O. S., Grashow, R., Gal, G., Braehler, E., & Weisskopf, M. G. (2014). Sexual and physical abuse in childhood is associated with depression and anxiety over the life course: systematic review and meta-analysis. *International Journal of Public Health, 59*, 359-372.

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychiatry, 167*, 47-55.

Liu, Q., Shono, M., Kitamura, T. (2009). Psychological well-being, depression, and anxiety in Japanese university students. *Depression and Anxiety, 26*, E99-E105.

Llabres, J., Bornas, X., Noguera, M., Lopez, A. M., & Barcelo, F. (2005). Is there chaos in the electrocardiogram of fearful flyers? Analyzing nonlinearity. *International Journal of Clinical and Health Psychology, 5*, 273-284.

Lonigan, C. J., & Vasey, M. W. (2009). Negative affectivity, effortful control, and attention to threat-relevant stimuli. *Journal of Abnormal Child Psychology, 37*, 387-399.

Luciano, M., Houlihan, L. M., Harris, S. E., Gow, A. J., Hayward, C., Starr, J. M., & Deary, I. J. (2010). Association of existing and new candidate genes for anxiety, depression and personality traits in older people. *Behavior Genetics, 40*, 518-532.

Lueken, U., Zierhut, K. C., Hahn, T., Straube, B., Kircher, T., Reif, A., … Domschke, K. (2016). Neurobiological markers predicting treatment response in anxiety disorders: a systematic review and implications for clinical application. *Neuroscience and Biobehavioral Reviews, 66*, 143-162.

Luna, B., Marek, S., Larsen, B., Tervo-Clemmens, B., & Chahal, R. (2015). An integrative model of the maturation of cognitive control. *Annual Review of Neuroscience, 38*, 151-170.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*, 434-445.

Manassis, K., Wylansly-Traynor, P., Farzan, N., Kleiman, V., Parker, K., & Sanford, M. (2010). The feelings club: randomized controlled evaluation of school-based CBT for anxious or depressive symptoms. *Depression and Anxiety, 27,* 945-952.

Masia-Werner, C., Storch, E. A., Pincus, D. B., Klein, R. G., Heimberg, R. G., & Leibowitz, M. R. (2003). The Liebowitz Social Anxiety Scale for Children and Adolescents: an initial psychometric investigation. *Journal of the American Academy of Child and Adolescent Psychiatry, 42,* 1076-1084.

McCarty, C. A., Violette, H. D., Duong, M. T., Cruz, R. A., & McCauley, E. (2013). A randomized trial of the Positive Thoughts and Action Program for depression among early adolescents. *Journal of Clinical Child and Adolescent Psychology, 42,* 554-563.

McDowell, R. D., Ryan, A., Bunting, B. P., O'Neill, S. M., Alonso, J., … Tomov, T. (2014). Mood and anxiety disorders across the adult lifespan: a European perspective. *Psychological Medicine, 44,* 707-722.

McEvoy, P. M., Nathan, P., & Norton, P. J. (2009). Efficacy of transdiagnostic treatments: a review of published outcome studies and future research directions. *Journal of Cognitive Psychotherapy, 23,* 20-33.

McEwen, B. S. (2006). Protective and damaging effects of stress mediators: central role of the brain. *Dialogues in Clinical Neuroscience, 8,* 368-381.

McLaughlin, K. A. (2011). The public health impact of major depression: a call for interdisciplinary prevention efforts. *Prevention Science, 12,* 361-371.

McLaughlin, K. A., & King, K. (2015). Developmental trajectories of anxiety and depression in early adolescence. *Journal of Abnormal Child Psychology, 43,* 311-323.

McLaughlin, K. A., Alves, S., & Sheridan, M. A. (2014). Vagal regulation and internalizing psychopathology among adolescents exposed to childhood adversity. *Developmental Psychobiology, 56,* 1036-1051.

McTeague, L. M., & Lang, P. J. (2012). The anxiety spectrum and the reflex physiology of defense: from ciscumscribed fear to broad distress. *Depression and Anxiety, 29,* 264-281.

McTeague, L. M., Lang, P. J., Wangelin, B. C., Laplante, M. C., & Bradley, M. M. (2012). Defensive mobilization in specific phobia: fear specificity, negative affectivity, and diagnostic prominence. *Biological Psychiatry, 72*, 8-18.

Menzies, L., Chamberlain, S. R., Laird, A. R., Thelen, S. M., Sahakian, B. J., & Bullmore, E. T. (2008). Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. *Neuroscience and Biobehavioral Reviews, 32*, 525-549.

Merikangas, K. R., Akiskal, H. S., Angst, J., Greenberg, P. E., Hirschfeld, R. M., Petukhova, M., & Kessler, R. C. (2007). Lifetime and 12 months prevalence of bipolar spectrum disorder in the National Comorbidity survey replication. *Archives of General Psychiatry, 64*, 542-543.

Merikangas, K. R., He, M. J., Burstein, M., Swanson, M. S. A., Avenevoli, S., Cui, M. L., . . . Swendsen, J. (2010). Lifetime prevalence of mental disorders in US adolescents: results from the national comorbidity study-adolescent supplement (NCS-A). *Journal of the American Academy of Child and Adolescent Psychiatry, 49*, 980.

Michl, L. C., McLaughlin, K. A., Shepherd, K., & Nolen-Hoeksema, S. (2013). Rumination as a mechanism linking stressful life events to symptoms of depression and anxiety: longitudinal evidence in early adolescents and adults. *Journal of Abnormal Psychology, 122*, 339-352.

Michopoulos, I., Douzenis, A., Kalkavoura, C., Christodoulou, C., Michalopoulou, P., Kalemi, G., … Lykouras, L. (2008). Hospital Anxiety and Depression Scale (HADS): validation in a Greek general hospital sample. *Annals of General Psychiatry, 7*, 4.

Milad, M. R., & Rausch, S. L. (2012). Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends in Cognitive Sciences, 16*, 43-51.

Miu, A. C., Heilman, R. M., & Miclea, M. (2009). Reduced heart rate variability and vagal tone in anxiety: trait versus state, and the effects of autogenic training. *Autonomic Neuroscience: Basic and Clinical, 145*, 99-103.

Mochcovitch, M. D., Freire, R. C., Garcia, R. F., & Nardi, A. E. (2014). A systematic review of fMRI studies in generalized anxiety disorder: evaluating its neural and cognitive basis. *Journal of Affective Disorders, 167*, 336-342.

Montoya, A., Bruins, R., Katzman, M. A., & Blier, P. (2016). The noradrenergic paradox: implications in the management of depression and anxiety. *Neuropsychiatric Disease and Treatment, 12*, 541-557.

Moon, E., Lee, S.-H., Kim, D.-H., & Hwang, B. (2013). Comparative study of heart rate variability in patients with schizophrenia, bipolar disorder, post-traumatic stress disorder, or major depressive disorder. *Clinical Psychopharmacology and Neuroscience, 11*, 137-143.

Moore, R. C., Depp, C. A., Wetherell, J. L., & Lenze, E. J. (2016). Ecological momentary assessment versus standard assessment instruments for measuring mindfulness, depressed mood, and anxiety among older adults. *Journal of Psychiatric Research, 75*, 116-123.

Moreno-Peral, P., Conejo-Ceron, S., Motrico, E., Rodriguez-Morejon, A., Fernandez, A., Garcia-Campayo, J., … Bellon, J. A. (2014). Risk factors for the onset of panic and generalised anxiety disorders in the general adult population: a systematic review of cohort studies. *Journal of Affective Disorders, 168*, 337-348.

Morey, R. A., Gold, A. L., LaBar, K. S., Beall, S. K., Brown, V. M., Haswell, C. C., … Mid-Atlantic, M. W. (2012). Amygdala volume changes in posttraumatic stress disorder in a large case-controlled veterans group. *Archives of General Psychiatry, 69*, 1169-1178.

Morin, A. J. S., Maiano, C., Nagengast, B., Marsh, H. W., Morizot, J., & Janosz, M. (2011). General Growth mixture analysis of adolescents' developmental trajectories of anxiety: the impact of untested invariance assumptions on substantive interpretations. *Structural Equation Modeling, 18*, 613-648.

Mota, N. P., Tsai, J., Sareen, J., Marx, B. P., Wisco, B. E., Harpaz-Rotem, I., … Pietrzak, R. H. (2016). High burden of subthreshold DSM-5 post-traumatic stress disorder in U.S. military veterans. *World Psychiatry, 15*, 185-186.

Mrazek, P. J., & Haggerty, R. J. (1994). *Reducing risks for mental disorders: frontiers for preventive intervention research*. Washington, DC: National Academy Press.

Mueller, E. M., Hofman, S. G., Santesso, D. L., Meuret, A. E., Bitran, S., & Pizzagalli, D. A. (2009). Electrophysiological evidence of attentional biases in social anxiety disorder. *Psychological Medicine, 39*, 1141-1152.

Mueller, S. C., Baudoncq, R., & de Schryver, M. (2015). The effect of parental loss on cognitive and affective interference in adolescent boys from a post-conflict region. *Journal of Adolescence, 42*, 11-19.

Muris, P., & Ollendick, T. H. (2015). Children who are anxious in silence: a review on selective mutism, the new anxiety disorder in DSM-5. *Clinical Child and Family Psychology Review, 18*, 151-169.

Muris, P., de Jong, P. J., & Engelen, S. (2004). Relationships between neuroticism, attentional control, and anxiety disorders symptoms in non-clinical children. *Personality and Individual Differences, 37*, 789-797.

Navarro-Mateu, F., Tormo, M. J., Salmeron, D., Vilagut, G., Navarro, C., Ruiz-Merino, G., … Alonso, J. (2015). Prevalence of mental disorders in the south-east of Spain, One of the European regions most affected by the economic crisis: The Cross-Sectional PEGASUS-Murcia Project. *PLoS One, 10*, e0137293.

Nelemans, S. A., Hale, W. W., III, Branje, S. J. T., Raaijmakers, Q. A. W., Frijns, T., van Lier, P. A. C., & Meeus, W. H. J. (2014). Heterogeneity in development of adolescent anxiety disorder symptoms in an 8-year longitudinal community study. *Development and Psychopathology, 26*, 181-202.

Newman, M. G., Shin, K. E., & Zuellig, A. R. (2016). Developmental risk factors in generalized anxiety disorder and panic disorder. *Journal of Affective Disorders, 206*, 94-102.

Nielsen, M. B., Tangen, T., Idsoe, T., Matthiesen, S. B., & Mageroy, N. (2015). Post-traumatic stress disorder as a consequence of bullying at work and at school. A literature review and meta-analysis. *Aggression and Violent Behavior, 21*, 17-34.

Nigg, J. T. (2006). Temperament and developmental psychopathology. *Journal of Child Psychology and Psychiatry, 47*, 395-422.

Nivard, M. G., Gage, S. H., Hottenga, J. J., Van Beijsterveldt, C. E. M., Abdellaoui, A., Bertels, M., … Middeldorp, C. M. (2017). Genetic overlap between schizophrenia and developmental psychopathology: longitudinal and multivariate polygenic risk prediction of common psychiatric traits during development. *Schizophrenia Bulletin, 43*, 1197-1207.

Nolen-Hoeksema, S., Wisco, B. E., & Lyubomirsky, S. (2008). Rethinking rumination. *Perspectives on Psychological Science, 3*, 400-424.

Norton, P. J., & Barrera, T. L. (2012). Transdiagnostic versus diagnosis-specific CBT for anxiety disorders: a preliminary randomized controlled non-inferiority trial. *Depression and Anxiety, 29*, 874-882.

Nuss, P. (2015). Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatric Disease and Treatment, 11*, 165-175.

Nusslock, R., Walden, K., & Harmon-Jones, E. (2015). Asymmetrical frontal cortical activity associated with differential risk for mood and anxiety disorder symptoms: an RDoC perspective. *International Journal of Psychophysiology, 98*, 249-261.

Nutt, D., Garcia-de Miguel, B., & Davis, S. J. C. (2008). Phenomenology of anxiety disorders. In R. J. Blanchard, D. C. Blanchard, G. Griebel and D. Nutt, *Handbook of anxiety and fear* (vol. 17, pp. 365-393). Amsterdam: Elsevier.

O'Connor, M., McDaniel, N., & Brady, W. J. (2008). The pediatric electrocardiogram. Part I: age-related interpretation. *American Journal of Emergency Medicine, 26*, 221-228.

Oerlemans, S., Mols, F., Nijziel, M. R., Zijlstra, W. P., Coebergh, J. W. W., & van de Poll-Franse, L. V. (2014). The course of anxiety and depression for patients with Hodgkin's lymphoma or diffuse large B cell lymphoma: a longitudinal study of the PROFILES registry. *Journal of Cancer Survivorship, 8*, 555-564.

Okasha, A. (2009). Would the use of dimensions instead of categories remove problems related to subthreshold disorders? *European Archives of Psychiatry and Clinical Neuroscience, 259*, S129-S133.

Olatunji, B. O., & Wolitzky-Taylor, K. B. (2009). Anxiety sensitivity and the anxiety disorders: a meta-analytic review and synthesis. *Psychological Bulletin, 135*, 974-999.

Olino, T. M., Klein, D. N., Lewinsohn, P. M., Rohde, P., & Seeley, J. R. (2010). Latent trajectory classes of depressive and anxiety disorders from adolescence to adulthood: Descriptions of classes and associations with risk factors. *Comprehensive Psychiatry, 51*, 224-235.

Ormel, J., Raven, D., van Oort, F., Hartman, C. A., Reijneveld, S. A., Veenstra, R., … Oldehinkel, A. J. (2015). Mental health in Dutch adolescents: a TRAILS report on prevalence, severity, age of onset, continuity and co-morbidity of DSM disorders. *Psychological Medicine, 45*, 345-360.

Otowa, T., York, T. P., Gardner, C. O., Kendler, K. S., & Hettema, J. M. (2014). The impact of childhood parental loss on risk for mood, anxiety and substance use disorders in a population-based sample of male twins. *Psychiatry Research, 220*, 404-409.

Palm, M. E., Elliott, R., McKie, S., Deakin, J. F., & Anderson, I. M. (2011). Attenuated responses to emotional expressions in women with generalized anxiety disorder. *Psychological Medicine, 41*, 1009-1018.

Palva, J. M., Zhigalov, A., Hirvonen, J., Korhonen, O., Linkenkaer-Hansen, K., & Palva, S. (2013). Neuronal long-range temporal correlations and avalanche dynamics are correlated with behavioral scaling laws. *Proceedings of the National Academy of Sciences, 110*, 3585-3590.

Parshall, C. G., Spray, J. A., Kalohn, J., & Davey, T. (2002). Considerations in computer-based testing. In *Practical considerations in computer-based testing* (pp. 1-12). New York, NY: Springer-Verlag.

Paulhus, D. L., & Vazire, S. (2007). The self-report method. In R. W. Robins, R. C. Fraley and R. F. Krueger (Eds.), *Handbook of research methods in personality psychology* (pp. 224-240). New York, NY: The Guilford Press.

Paus, T., Keshavan, M., & Giedd, J. N. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews Neuroscience, 9*, 947-957.

Pearson, S. R., Alkon, A., Treadwell, M., Wolff, B., Quirolo, K., & Boyce, W. T. (2005). Autonomic reactivity and clinical severity in children with sickle cell disease. *Clinical Autonomic Research, 15*, 400-407.

Peltola, M. J., Bakermans-Kranenburg, M. J., Alink, L. R. A., Huffmeijer, R., Biro, S., & van Ijzendoorn, M. H. (2014). Resting frontal EEG asymmetry in children: meta-analyses of the effects of psychosocial risk factors and associations with internalizing and externalizing behavior. *Developmental Psychobiology, 56*, 1377-1389.

Perez-Edgar, K., Bar-Haim, Y., McDermott, J. M., Chronis-Tuscano, A., Pine, D. S., & Fox, N. A. (2010). Attention biases to threat and behavioral inhibition in early childhood shape adolescent social withdrawal. *Emotion, 10,* 349-357.

Petrowski, K., Wichmann, S., Siepmann, T., Bornstein, S. R., & Siepmann, M. (2017). Effects of mental stress induction on heart rate variability in patients with panic disorder. *Applied Psychophysiology and Biofeedback, 42*, 85-94.

Petrowski, K., Wintermann, G. B., Schaarschmidt, M., Bornstein, S. R., & Kirschbaum, C. (2013). Blunted salivary and plasma cortisol response in patients with panic disorder under psychosocial stress. *International Journal of Psychophysiology, 88*, 35-39.

Pham, X., Sun, C., Chen, X., van den Oord, E. J. C. G., Neale, M. C., Kendler, K. S., & Hettema, J. M. (2009). Association study between GABA receptor genes and anxiety spectrum disorders. *Depression and Anxiety, 26,* 998-1003.

Pittig, A., Arch, J. J., Lam, C. W. R., & Craske, M. G. (2013). Heart rate and heart rate variability in panic, social anxiety, obsessive–compulsive, and generalized anxiety disorders at baseline and in response to relaxation and hyperventilation. *International Journal of Psychophysiology, 87,* 19-27.

Pluess, M., Conrad, A., & Wilhelm, F. H. (2009). Muscle tension in generalized anxiety disorder: a critical review of the literature. *Journal of Anxiety Disorders, 23*, 1-11.

Porges, S. W. (2001). The polyvagal theory: phylogenetic substrates of a social nervous system. *International Journal of Psychophysiology, 42,* 123-146.

Proust-Lima, C., Sene, M., Taylor, J. M., & Jacqmin-Gadda, H. (2014). Joint latent class models for longitudinal and time-to-event data: a review. *Statistical Methods in Medical Research, 23*, 74 90.

Queen, A. H., Barlow, D. H., & Ehrenreich-May, J. (2014). The trajectories of adolescent anxiety and depressive symptoms over the course of a transdiagnostic treatment. *Journal of Anxiety Disorders, 28,* 511-521.

Radzik, M., Sherer, S., & Neinstein, L. S. (2008). Psychosocial development in normal adolescents. In L. Neinstein (Ed.), *Handbook of adolescent healthcare: a practical guide* (5th ed., pp. 27-44). Philadelphia, PA: Lipppincott, Williams & Wilkins.

Rapee, R. M. (2014). Preschool environment and temperament as predictors of social and nonsocial anxiety disorders in middle adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry, 53*, 320-328.

Rapee, R. M., & Coplan, R. J. (2010). Conceptual relations between anxiety disorder and fearful temperament. In H. Gazelle & K. H. Rubin (Eds.), *Social anxiety in childhood: bridging developmental and clinical perspectives. New Directions for Child and Adolescent Development* (vol. 127, pp. 17-31). San Francisco, CA: Jossey-Bass.

Rapee, R. M., & Hayman, K. (1996). The effects of video feedback on the self-evaluation of performance in socially anxious subjects. *Behaviour, Research and Therapy, 34*, 315-322.

Reeb-Sutherland, B. C., Vanderwert, R. E., Degnan, K. A., Marshall, P. J., Perez-Edgar, K., Chronis-Tuscano, A., Pine, D. S., & Fox, N. A. (2009). Attention to novelty in behaviorally inhibited adolescents moderates risk for anxiety. *Journal of Child Psychology and Psychiatry, 50*, 1365-1372.

Reeb-Sutherland, B. C., Williams, L. R., Degnan, K. A., Perez-Edgar, K., Chronis-Tuscano, A., … Fox, N. A. (2015). Identification of emotional facial expressions among behaviorally inhibited adolescents with lifetime anxiety disorders. *Cognition and Emotion, 39*, 372-382.

Reiss, S., & McNally, R. J. (1985). The expectancy model of fear. In S. Reiss and R. R. Bootzin (Eds.), *Theoretical issues in behavior therapy* (pp. 107-121). London: Academic Press.

Roberts, R. E., Fisher, P. W., Turner, J. B., & Tang, M. (2015). Estimating the burden of psychiatric disorders in adolescence: the impact of subthreshold disorders. *Social Psychiatry and Psychiatric Epidemiology, 50*, 397-406.

Rodebaugh, T. L. (2004). I might look OK, but I'm still doubtful, anxious, and avoidant: the mixed effects of enhanced video feedback on social anxiety symptoms. *Behaviour, Research and Therapy, 42*, 1435-1451.

Rose, M., & Devine, J. (2014). Assessment of patient-reported symptoms of anxiety. *Dialogues in Clinical Neuroscience, 16*, 197-211.

Roth, W. T. (2005). Physiological markers for anxiety: panic disorder and phobias. *International Journal of Psychophysiology, 58*, 190-198.

Rothbart, M. K. (2007). Temperament, development, and personality. *Current Directions in Psychological Science, 16*, 207-212.

Rubin, K. H., Coplan, R. J., Bowker, J. C., & Menzer, M. (2011). Social withdrawal and shyness. In P. K. Smith & C. Hart (Eds.), *Blackwell's handbook of childhood social development* (2nd ed, pp. 434-452). Guichester: John Wiley & Sons, Ltd.

Rucci, P., Gherardi, S., Tansella, M., Piccinelli, M., Berardi, D., Bisoffi, G., … Pini, S. (2003). Subthreshold psychiatric disorders in primary care: prevalence and associated characteristics. *Journal of Affective Disorders, 76*, 171-181.

Salim, S., Chugh, G., & Asghar, M. (2012). Inflammation in anxiety. In R. Donev (Ed.), *Advances in Protein Chemistry and Structural Biology* (vol. 88, doi:10.1016/B978-0-12-398314-5.00001-5). Oxford: Elsevier, Inc.

Santoro, M. L., Moretti, P. N., Pellegrino, R., Gadelha, A., Abilio, V. C., Hayashi, M. A. F., … Hakonarson, H. (2016). A current snapshot of common genomic variants contribution in psychiatric disorders. *American Journal of Medical Genetics – Part B. Neuropsychiatric Genetics, 171B*, 997-1005.

Savage, J. E., Sawyers, C., Roberson-Nay, R., & Hettema, J. M. (2017). The genetics of anxiety-related negative valence system traits. *American Journal of Medical Genetics – Part B. Neuropsychiatric Genetics, 174B*, 156-177.

Schienle, A., Ebner, F., & Schäfer, A. (2011). Localized gray matter volume abnormalities in generalized anxiety disorder. *European Archives of Psychiatry and Clinical Neuroscience, 261*, 303-307.

Schmitz, J., Kramer, M., Tuschen-Caffer, B., Heinrichs, N., & Blechert, J. (2011). Restricted autonomic flexibility in children with social phobia. *Journal of Child Psychology and Psychiatry, 52*, 1203-1211.

Schmitz, J., Tuschen-Caffer, B., Wilhelm, F. H., & Blechert, J. (2013). Taking a closer look: autonomic dysregulation in socially anxious children. *European Child and Adolescence Psychiatry, 22*, 631-640.

Schuldberg, D. (2015). What is optimum variability? *Nonlinear Dynamics, Psychology, and Life Sciences, 19*, 553-568.

Self-Brown, S., Lai, B., Harbin, S., & Kelley, M. L. (2014). The impact of parental posttraumatic stress disorder symptom trajectories on the long-term outcomes of youth following Hurricane Katrina. *International Journal of Public Health, 59*, 957-965.

Seligman, L. D., & Gahr, J. L. (2013). Course and outcome of child and adolescent anxiety. In C. A. Essau and T. H. Ollendick (Eds.), *The treatment of childhood and adolescent anxiety* (pp. 43-68). Malden, MA: John Wiley & Sons, Ltd.

Shaffer, F., McCraty, R., & Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology, 5*, 1040.

Shankman, S. A., Klein, D. N., Lewinsohn, P. M., Seeley, J. R., & Small, J. W. (2008). Family study of subthreshold psychopathology in a community sample. *Psychological Medicine, 38*, 187-198.

Shankman, S. A., Lewinsohn, P. M., Klein, D. N., Small, J. W., Seeley, J. R., & Altman, S. E. (2009). Subthreshold conditions as precursors for full syndrome disorders: a 15-year longitudinal study of multiple diagnostic classes. *Journal of Child Psychology and Psychiatry, 50*, 1485-1494.

Shiffman, S., Stone, A. A., & Hufford, M. R. (2008). Ecological momentary assessment. *Annual Review of Clinical Psychology, 4*, 1-32.

Siess, J., Blechert, J., & Schmitz, J. (2013). Psychophysiological arousal and biased perception of bodily anxiety symptoms in socially anxious children and adolescents: a systematic review. *European Child and Adolescent Psychiatry, 23*, 127-142.

Simpson, H. B. (2012). The RDoC project: a new paradigm for investigating the pathophysiology of anxiety. *Depression and Anxiety, 29*, 251-252.

Skinner, E. A., & Zimmer-Gembeck, M. J. (2007). The development of coping. *Annaul Review of Psychology, 58*, 119-144.

Smith, J. C., Bradley, M. M., & Lang, P. J. (2005). State anxiety and affective physiology: effects of sustained exposure to affective pictures. *Biological Psychology, 69*, 247-260.

Snyder H., Young J., & Hankin B. (2017). Strong homotypic continuity in common psychopathology-, internalizing and externalizing-specific factors over time in adolescents. *Clinical Psychological Science, 5*, 98-110.

So, H.-C., & Sham, P. C. (2017). Exploring the predictive power of polygenic scores derived from genome-wide association studies: a study of 10 complex traits. *Bioinformatics, 33*, 886-892.

Soto, C. J., & Tackett, J. L. (2015). Personality traits in childhood and adolescence: structure, development, and outcomes. *Current Directions in Psychological Sciences, 24*, 358-362.

Sportel, B. E., Nauta, M. H., de Hullu, E., de Jong, P., & Hartman, C. A. (2011). Behavioral inhibition and attentional control in adolescents: robust relationships with anxiety and depression. *Journal of Child and Family Studies, 20*, 149-156.

Stockings, E. A., Degenhardt, L., Dobbins, T., Lee, Y. Y., Erskine, H. E., Whiteford, H. A., & Patton, G. (2016). Preventing depression and anxiety in young people: a review of the joint efficacy of universal, selective and indicated prevention. *Psychological Medicine, 46*, 11-26.

Stuhldreher, N., Leibing, E., Leichsenring, F., Beutel, M. E., Herpertz, S., … Konig, H.-H. (2014). The cost of social anxiety disorder: the role of symptom severity and comorbidities. *Journal of Affective Disorders, 165*, 87-94.

Sturmberg, J. P., Bennet, J. M., Picard, M., & Seeley, A. J. E. (2015). The trajectory of life. Decreasing physiological network complexity through changing fractal patterns. *Frontiers in Physiology, 6*, 169.

Susa, G., Pitica, I., Benga, O., & Miclea, M. (2012). The self regulatory effect of attentional control in modulating the relationship between attentional biases toward threat and anxiety symptoms in children. *Cognition and Emotion, 26*, 1069-1083.

Tancer, M. E., Stein, M. B., & Uhde, T. W. (1994). Lactic acid response to caffeine in panic disorder: comparison with social phobics and normal controls. *Anxiety, 1*, 138-140.

Tapanainen, J. M., Thomsen, P. E. B., Kober, L., Torp-Pedersen, C., Makikallio, T. H., Still, A.-M., … Huikuri, H. V. (2002). Fractal analysis of heart rate variability and mortality after an acute myocardial infarction. *American Journal of Cardiology, 90*, 347-352.

Tendais, I., Costa, R., Conde, A., & Figueiredo, B. (2014). Screening for depression and anxiety disorders from pregnancy to postpartum with the EPDS and STAI. *Spanish Journal of Psychology, 17*, 1-9.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart–brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*, 81-88.

Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences, 1088*, 361-372.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., 3rd, & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*, 747-756.

Thibodeau, R., Jorgensen, R. S., & Kim, S. (2006). Depression, anxiety, and resting frontal EEG asymmetry: a meta-analytic review. *Journal of Abnormal Psychology, 115*, 715-729.

Thoeringer, C. K., Ripke, S., Unschuld, P. G., Lucae, S., Ising, M., Bettecken, T., …Erhardt, A. The GABA transporter 1 (SLC6A1): a novel candidate gene for anxiety disorders. *Journal of Neural Transmission, 116*, 649-657.

Tiihonen, J., Kuikka, J., Bergstrom, K., Lepola, U., Koponen, H., & Leinonen, E. (1997). Dopamine reuptake site densities in patients with social phobia. *American Journal of Psychiatry, 154*, 239-242.

Topper, M., Emmelkamp, P. M. G., Watkins, E., & Ehring, T. (2017). Prevention of anxiety disorders and depression by targeting excessive worry and rumination in adolescents and young adults: a randomized controlled trial. *Behaviour Research and Therapy, 90*, 123-136.

Tortella-Feliu, M., Balle, M., & Sese, A. (2010). Relationships between negative affectivity, emotion regulation, anxiety, and depressive symptoms in adolescents as examined through structural equation modeling. *Journal of Anxiety Disorders, 24*, 686-693.

Tortella-Feliu, M., Morillas-Romero, A., Balle, M., Bornas, X., Llabres, J., & Pacheco-Unguetti, A. P. (2014). Attentional control, attentional network functioning, and emotion regulation styles. *Cognition and Emotion, 28*, 769-780.

Trzaskowski, M., Zavos, H. M. S., Haworth, C. M. A., Plomin, R., & Eley, T. C. (2012). Stable genetic influence on anxiety-related behaviours across middle childhood. *Journal of Abnormal Child Psychology, 40*, 85-94.

Tsui, T. Y. L., Lahat, A., &Schmidt, L. A. (2016). Linking temperamental shyness and social anxiety in childhood and adolescence: moderating inluences of sex and age. *Child Psychiatry and Human Development*. doi: 10.1007/s10578-016-0702-z

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Review Neuroscience, 10*, 397-409.

Vaitl, D. & Schandry, R. (1995). *From the heart to the brain: the psychophysiology of circulation – brain interaction.* New York, NY: Frankfurt am Main.

Van de Mortel, T. F. (2008). Faking it: social desirability response bias in self-report research. *Australian Journal of Advanced Nursing, 25*, 40-48.

Van Oorst, F. V. A., Greaves-Lord, K., Ormel J., Verhulst, F. C., & Huizink, A. C. (2011). Risk indicators of anxiety throughout adolescence: the TRAILS study. *Depression and Anxiety, 28*, 485-494.

Van Os, J. (2013). The dynamics of subthreshold psychopathology: implications for diagnosis and treatment. *American Journal of Psychiatry, 170*, 695-698.

Van Tuijl, L. A., de Jong, P. J., Sportel, B. E., de Hullu, E., & Nauta, M. H. (2014). Implicit and explicit self-esteem and their reciprocal relationship with symptoms of depression and social anxiety: a longitudinal study in adolescents. *Journal of Behavior Therapy and Experimental Psychiatry, 45*, 113-121.

Vanderhasselt, M. A., Brose, A., Koster, E. H., & De Raedt, R. (2016). Co-variation between stressful events and rumination predicts depressive symptoms: an eighteen months prospective design in undergraduates. *Behaviour Research and Therapy, 87*, 128-133.

Vervoort, L., Wolters, L. H., Hogendoorn, S. M., de Haan, E., Boer, F., & Prins, P. J. M. (2010). Sensitivity of Gray's behavioral inhibition system in clinically anxious and non-anxious children and adolescents. *Personality and Individual Differences, 48*, 629-633.

Viana, A. G., Palmer, C. A., Zvolensky, M. J., Alfano, C. A., Dixon, L. J., & Raines, E. M. (2017). Children's behavioral inhibition and anxiety disorder symptom severity: the role of individual differences in respiratory sinus arrhythmia. *Behaviour Research and Therapy, 93*, 38-46.

Vogelzangs, N., Beekman, A. T. F., de Jonge, P., & Penninx, B. W. J. H. (2013). Anxiety disorders and inflammation in a large adult cohort. *Translational Psychiatry, 3*, e249.

Walz, L. C., Nauta, M. H., & aan het Rot, M. (2014). Experience sampling and ecological momentary assessment for studying the daily lives of patients with anxiety disorders: a systematic review. *Journal of Anxiety Disorders, 28*, 925-937.

Waszczuk, M. A., Zavos, H. M. S., Gregory, A. M., & Eley, T. C. (2016). The stability and change of etiological influences on depression, anxiety symptoms and their co-occurrence across adolescence and young adulthood. *Psychological Medicine, 46*, 161-175.

Watkins, E. R. (2008). Constructive and unconstructive repetitive thought. *Psychological Bulletin, 134*, 163-206.

Wauthia, E., & Rossignol, M. (2016). Emotional processing and attention control impairments in children with anxiety: an integrative review of event-related potentials findings. *Frontiers in Psychology, 3*, 562.

Weems, C. F. (2008). Developmental trajectories of childhood anxiety: identifying continuity and change in anxious emotion. *Developmental Review, 28*, 488-502.

Weik, U., Herforth, A., Kolb-Bachofen, V., & Deinzer, R. (2008). Acute stress induces proinflammatory signaling at chronic inflammation sites. *Psychosomatic Medicine, 70*, 906-912.

Werner, G. (2010). Fractals in the nervous system: conceptual implications for theoretical neuroscience. *Frontiers in Physiology, 1*, 15.

Werner-Seidler, A., Perry, Y., Calear, A. L., Newby, J. M., & Christensen, H. (2017). School-based depression and anxiety prevention programs for Young people: a systematic review and meta-analysis. *Clinical Psychology Review, 51*, 30-47.

West, B. J. (2006). *Where Medicine went wrong: rediscovering the path to complexity*. Singapore: World Scientific.

Wiklund, M., Malmgren-Olsson, E.-B., Ohman, A., Bergstrom, E., & Fjellman-Wiklund, A. (2012). Subjective health complaints in older adolescents are related to perceived stress, anxiety and gender – a cross-sectional school study in Northern Sweden. *BMC Public Health, 12*, 993.

Wilhelm, F. H., Trabert, W., & Roth, W. T. (2001). Physiologic instability in panic disorder and generalized anxiety disorder. *Biological Psychiatry, 49*, 596-605.

Wolitzky-Taylor, K., Dour, H., Zinbarg, R., Mineka, S. Vrshek-Schallhorn, S., Epstein, A., ... Craske, M. G. (2014). Experiencing core symptoms of anxiety and unipolar mood disorders in late adolescence predicts disorder onset in early adulthood. *Depression and Anxiety, 31*, 207-213.

World Health Organization. (2004). *Prevention of mental disorders. Effective interventions and policy options: summary report*. Geneva: WHO Press.

World Health Organization. (2011). *Outcome evaluation summary report: WHO/UNODC global initiative on primary prevention of substance abuse*. Geneva: WHO Press.

World Health Organization. (2017). Risk factors. Retrieved from http://www.who.int/topics/risk_factors/en/

Woud, M. L., Becker, E. S., Rinck, M., Harmer, C. J., & Reinecke, A. (2016). Assessment of automatic associations with bodily sensations and agoraphobic situations in panic disorder. *Journal of Behavior Therapy and Experimental Psychiatry, 52*, 105-109.

Yang, A. C., & Tsai, S.-J. (2013). Is mental illness complex? From behavior to brain. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 45*, 253-257.

Yeragani, V. K., Nadella, R., Hinze, B., Yeragani, S., & Jampala, V. C. (2000). Nonlinear measures of heart period variability: decreased measures of symbolic dynamics in patients with panic disorder. *Depression and Anxiety, 12*, 67-77.

Zammit, S., Kounali, D., Cannon, M., David, A. S., Gunnell, D., Heron, J., … Lewis, G. (2013). Psychotic experiences and psychotic disorders at age 18 in relation to psychotic experiences at age 12 in a longitudinal population-based cohort study. *American Journal of Psychiatry, 170,* 742-750.

Zavaglia, E., & Bergeron, L. (2017). Systematic review of comorbidity between dsm disorders and depression according to age and sex in youth. *Canadian Psychology, 58*, 124-139.

Zavos, H. M. S., Eley, T. C., & Gregory, A. M. (2013). Genetic and environmental influences on child and adolescent anxiety. In C. A. Essau and T. H. Ollendick (Eds.), *The treatment of childhood and adolescent anxiety* (pp. 69-88). Malden, MA: John Wiley & Sons, Ltd.

Zijlmans, M. A. C., Riksen-Walraven, J. M., & de Weerth, C. (2015). Associations between maternal prenatal cortisol concentrations and child outcomes: a systematic review. *Neuroscience and Biobehavioral Reviews, 53*, 1-24.

Zlotnik, C., Franklin, C. L., & Zimmerman, M. (2002). Does subthreshold posttraumatic stress disorder have any clinical significance? *Comprehensive Psychiatry, 43*, 413-419.

Annexes 5

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Dr. Maria Balle, as a co-author of the following articles:

- De la Torre-Luque, A., Fiol-Veny, A., Nelemans S. A., Balle, M., & Bornas, X. Anxiety from early to middle adolescence: Developmental trajectories and associations with temperamental factors. Currently under review in *Journal of Abnormal Child Psychology*.
- De la Torre-Luque, A., Bornas, X., Balle, M., & Fiol-Veny, A. (2016). Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. Neuroscience & Biobehavioral Reviews, 68, 410-422. doi: 10.1016/j.neubiorev.2016.05.023
- De la Torre-Luque, A., Fiol-Veny, A., Balle, M., & Bornas, X. (2016). Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment. International Journal of Clinical and Health Psychology, 16, 287-294. doi: 10.1016/j.ijchp.2016.04.002
- De la Torre-Luque, A., Fiol-Veny, A., Bornas, X., Balle, M., & Llabres, J. (2017). Impaired cardiac profile in adolescents with an increasing trajectory of anxiety when confronting an acute stressor. European Child and Adolescent Psychiatry. doi: 10.1007/s00787-017-1009-8
- Bornas, X., De la Torre-Luque, A., Fiol-Veny, A., & Balle, M. (2017). Trajectories of anxiety symptoms in adolescents: Testing the model of emotional inertia. International Journal of Clinical and Health Psychology, 17, 192-196. doi: 10.1016/j.ijchp.2017.01.002

I declare to accept that Mr. Alejandro de la Torre-Luque presents the abovementioned articles as part of his doctoral thesis. Therefore, the mentioned article cannot be part of any other doctoral thesis.

And for all intents and purposes, hereby I sign this document:

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- De la Torre-Luque, A., Bornas, X., Balle, M., & Fiol-Veny, A. (2016). Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. Neuroscience & Biobehavioral Reviews, 68, 410-422. doi: 10.1016/j.neubiorev.2016.05.023
- De la Torre-Luque, A., Fiol-Veny, A., Balle, M., & Bornas, X. (2016). Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment. International Journal of Clinical and Health Psychology, 16, 287-294. doi: 10.1016/j.ijchp.2016.04.002
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I declare to accept that Mr. Alejandro de la Torre-Luque presents the abovementioned articles as part of his doctoral thesis. Therefore, the mentioned article cannot be part of any other doctoral thesis.

And for all intents and purposes, hereby I sign this document:

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- De la Torre-Luque, A., Bornas, X., Balle, M., & Fiol-Veny, A. (2016). Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. Neuroscience & Biobehavioral Reviews, 68, 410-422. doi: 10.1016/j.neubiorev.2016.05.023
- De la Torre-Luque, A., Fiol-Veny, A., Balle, M., & Bornas, X. (2016). Heartbeat scaling in early adolescents: Its association with anxiety symptoms and sensitivity to punishment. International Journal of Clinical and Health Psychology, 16, 287-294. doi: 10.1016/j.ijchp.2016.04.002
- De la Torre-Luque, A., Fiol-Veny, A., Bornas, X., Balle, M., & Llabres, J. (2017). Impaired cardiac profile in adolescents with an increasing trajectory of anxiety when confronting an acute stressor. European Child and Adolescent Psychiatry. doi: 10.1007/s00787-017-1009-8
- Bornas, X., De la Torre-Luque, A., Fiol-Veny, A., & Balle, M. (2017). Trajectories of anxiety symptoms in adolescents: Testing the model of emotional inertia. International Journal of Clinical and Health Psychology, 17, 192-196. doi: 10.1016/j.ijchp.2017.01.002

I declare to accept that Mr. Alejandro de la Torre-Luque presents the abovementioned articles as part of his doctoral thesis. Therefore, the mentioned article cannot be part of any other doctoral thesis.

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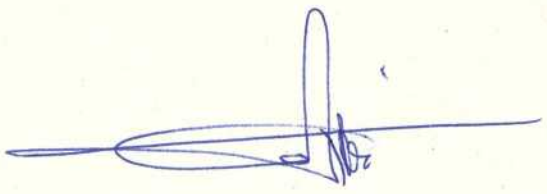

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I declare to accept that Mr. Alejandro de la Torre-Luque presents the abovementioned article as main author and as part of his doctoral thesis. Therefore, the mentioned article cannot be part of any other doctoral thesis.

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I declare to accept that Mr. Alejandro de la Torre-Luque presents the abovementioned article as main author and as part of his doctoral thesis. Therefore, the mentioned article cannot be part of any other doctoral thesis.

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