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HRV patterns associated with different affect regulation systems: sex differences in adolescents

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Abstract:	<p>Evolutionary perspectives of human behavior propose the existence of three emotion regulation systems (i.e., threat, drive and soothing systems). An unbalanced functioning of the systems represents greater risk for emotion dysregulation and psychopathology. In recent years, heart rate variability (HRV) has been reported as an accurate index of emotion regulation, and although adolescence is characterized by multiple neurophysiological, psychological and social changes, there is no study exploring the HRV patterns of each emotion regulation system in this developmental stage. In Study 1, a standardized procedure (SP) aiming to elicit the three different systems was developed and validated by experts (n = 14) and community adolescents (n = 31). In study 2, differences in HRV patterns across the three emotion regulation systems and across sex, were investigated in a sample of community adolescents (n = 155; 70 males), aged between 14 and 18 years old. Results showed that the threat and drive systems were associated with decreases in HRV, while the soothing system was associated with decreased heart rate. Sex differences were found for the activation of the threat system: while males maintained a decreasing trend in HRV indexes, from resting to recovery, females did not show a decrease in HRV during the activation of this system.</p>
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Highlights

- First test to the Emotion Regulation Systems Theory in adolescents.
- System specific physiological patterns were explored (i.e., threat/drive/soothing).
- A standardized procedure was developed, and HR/HRV was assessed in all phases.
- Findings support system specific physiological and emotional patterns.
- For the threat system, sex differences in HRV were found.

HRV patterns associated with different affect regulation systems: sex differences in adolescents

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My coauthors and I do not have any interests that might be interpreted as influencing the research and APA ethical standards were followed when conducting the study, which was approved by the ethic entity in Portugal that regulates studies conducted in school settings and by institutions boards.

Abstract

1 Evolutionary perspectives of human behavior propose the existence of three emotion regulation
2 systems (i.e., threat, drive and soothing systems). An unbalanced functioning of the systems
3 represents greater risk for emotion dysregulation and psychopathology. In recent years, heart
4 rate variability (HRV) has been reported as an accurate index of emotion regulation, and
5 although adolescence is characterized by multiple neurophysiological, psychological and social
6 changes, there is no study exploring the HRV patterns of each emotion regulation system in this
7 developmental stage. In Study 1, a standardized procedure (SP) aiming to elicit the three
8 different systems was developed and validated by experts (n = 14) and community adolescents
9 (n = 31). In study 2, differences in HRV patterns across the three emotion regulation systems
10 and across sex, were investigated in a sample of community adolescents (n = 155; 70 males),
11 aged between 14 and 18 years old. Results showed that the threat and drive systems were
12 associated with decreases in HRV, while the soothing system was associated with decreased
13 heart rate. Sex differences were found for the activation of the threat system: while males
14 maintained a decreasing trend in HRV indexes, from resting to recovery, females did not show a
15 decrease in HRV during the activation of this system.

16 Overall, physiological correlates of each specific emotion regulation system corroborate the
17 theoretical assumptions. Moreover, a SP able to trigger each system independently while
18 measuring physiological data is now available and can be used in future research.

Keywords

19 Affect Regulation Systems Theory; Heart Rate Variability; Adolescence; Sex Differences;
20 Autonomic Nervous System

1. Introduction

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2 Evolutionary psychology has proposed at least three basic life tasks that mammals are
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4 confronted with: to detect and pick up on threats quickly and select a response; to seek out,
5
6 acquire, and maintain resources needed in order to survive and prosper; and to downregulate
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8 arousal and inhibit resource seeking during times of affiliation and goal satisfaction (Gilbert
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10 2005, 2020; Porges, 2007). Drawing on research in neuroscience (Depue & Morrone-
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12 Strupinsky, 2005; LeDoux, 1998; Panksepp, 1998), Gilbert (2010) proposed the existence of
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14 three specialized emotion regulation systems, serving these adaptive functions: the threat, drive
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16 and soothing systems. The former yields negative affect (NA) whereas the latter two promote
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18 different types of positive affect (PA). Although emotion systems often blend, different types of
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20 emotions regulate different psychophysiological processes central to the function of the system.
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22 Evolution builds brains that are prepared for the quick detection of threats, producing “better
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24 safe than sorry” cognitive and behavioral styles. Different circuits regulate different decisions
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26 such as whether threat is immediate, in the future, or whether it is avoidable (Mobbs et al.,
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28 2009). Threat processes are very susceptible to early learning and classical conditioning and
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30 have major impacts on the degree to which individuals trust their environments (Mobbs et al.,
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32 2009; Mobbs et al., 2020). Based on this evolutionary approach, the emotion regulation systems
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34 theory also underpins psychotherapeutic approaches such as the Compassion Focused Therapy
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36 (CFT) which aims to improve the capacity for emotion regulation through the stimulation of the
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38 soothing system as a way to promote a more balanced autonomic nervous system (Gilbert,
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40 2010). When working in an interactive, interdependent, and balanced way, the three systems
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42 enable individuals to adopt adaptive and flexible behaviors and use different emotional
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44 regulation strategies (Gilbert, 2019, 2020; Rodrigues et al., 2009).
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51 The threat system, characterized by negative affect (e.g., anxiety, anger, sadness, shame), is
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53 responsible for the quick detection of threats, promoting the activation of appropriate defensive
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55 strategies (e.g., fight, flight or freeze). This system is associated with the activity of specific
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57 brain structures, such as the amygdala, and the hypothalamic-pituitary-adrenal axis (HPA;
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59 Mobbs et al., 2020). Hormones such as adrenaline, noradrenaline and cortisol (Richardson et al.,
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2016; Rodrigues et al., 2009) and the activation of the sympathetic nervous system (SNS) are associated with behaviours that require movement, and dorsal vagal parasympathetic nervous system when defences involve immobility (Porges, 2007). In general, when facing threats that require activated behaviours and the release of energy, individuals show elevated SNS activity, and subsequent increased heart rate (HR). The hyperactivation of the threat system is associated with decreased executive functioning, increased rumination, social isolation and with increased vulnerability to the development of psychopathology (Mobbs et al., 2020; Rodrigues et al., 2009).

The drive and resource system is a positive affect regulation system with the function of activating and mobilizing the organism for the search and acquisition of incentives and resources, as well as guiding the individual towards the satisfaction of biological needs (i.e., reproduction, food, shelter, social status; Depue & Morrone-Strupinsky, 2005; Panksepp, 1998). The activation of this system is associated with happiness, vitality and excitement, motivating the individual to fulfill desires and necessities essential for survival. At the physiological level, the drive system is associated with the action of dopaminergic circuits and SNS activity (Richardson et al., 2016), allowing HR to increase. When triggered in a balanced manner, this system enables individuals to pursue and accomplish important and relevant life goals (Gilbert, 2020).

Finally, the soothing system, part of the rest and digest system, also a positive emotion regulation system, is associated with increased compassion (Di Bello et al., 2020), with feelings of safeness, affiliation and prosocial behaviors that can regulate the activation of both the drive and the threat systems (Depue & Morrone-Strupinsky, 2005; Gilbert, 2020; Porges, 2007). Research suggests this is an affect regulation system “in its own right” (Armstrong III et al., 2020). The soothing system is activated by the perception of safeness and connection with others and stimulates pre-frontal cortex activity related to the inhibition of continuous threat perception (Armstrong III et al., 2020; Gilbert 2020; Petrocchi & Cheli, 2019). Prosocial interactions with the environment and others can also emerge while the soothing system is active (Depue & Morrone-Strupinsky, 2005; Gilbert, 2020). At the physiological level, the

1 triggering of this system is associated with PNS activity, endorphins, and oxytocin circuits,
2 allowing HR to decrease and the stress response to be inhibited (Depue & Morrone-Strupinsky,
3 2005; McCorry, 2007). The activation of the soothing system is able to regulate both the
4 emotional and behavioral outputs of the other systems via increased activity of the vagal
5 parasympathetic system and increasing heart rate variability (HRV; Petrocchi et al., 2017;
6 Petrocchi & Cheli, 2019; Porges, 2017).
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13 Research on physiological correlates of affect regulation has shown that vagally-mediated
14 heart rate variability (vmHRV) is an accurate marker of the autonomic activity/flexibility by
15 reflecting parasympathetic nervous system activity (vagus nerve influence) on the cardiac
16 output (Malik, 1996; Porges, 2007; Thayer et al., 2009). The vagus, the 10th cranial nerve, is the
17 main nerve of the PNS, innervating multiple organs within the body. It acts as a “brake” on the
18 sympathetic nervous system and can rapidly exert an inhibitory influence on the cardiac activity,
19 decreasing HR and increasing HRV (Porges, 2017). Therefore, vagally-mediated HRV is
20 considered an index of the autonomic nervous system (ANS) activity and a marker of the
21 organism ability to regulate and use adaptive emotional responses (Porges, 2007; Thayer et al.,
22 2012). The vagal control of the heart inhibits sympathetic activity, increases HRV and can
23 reduce the stress response of the HPA axis, allowing for stable and adaptive interactions in
24 social relations (Appelhans & Luecken, 2006). In contrast, maladaptive emotion regulation
25 strategies, characterized by a reduced activity of the soothing system, lower HRV, and the
26 hyperactivity of the threat and drive systems, are associated with undesired emotional
27 responses, pathological behavioral patterns and a wide range of psychopathologies (Gilbert,
28 2014; Ribeiro da Silva et al., 2015).
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49 Although previous research on the tripartite model has explored the distinction between
50 negative affect (associated with the threat system) and the different types of positive affect
51 (activated positive affect, linked to the drive system; and relaxed/safe positive affect, linked to
52 the soothing system (Armstrong III et al., 2020; Gilbert et al., 2008; Kelly et al., 2012), these
53 studies only reported self-report measures and are mostly conducted with adult samples.
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60 Therefore, a more in-depth exploration of the physiological patterns of the three emotion
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1 regulation systems is needed, including the systems' activity during other developmental stages,
2 such as adolescence. In fact, the acquisition of adaptive emotion regulation strategies is
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4 considered of major relevance in early stages of development such as adolescence (McLaughlin
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6 et al., 2011), characterized by multiple neurophysiological, psychological and social changes
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8 (Desatnik et al., 2017; Theurel & Gentaz, 2018). Moreover, in this complex developmental
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10 stage, the sex of the individual seems to play a relevant role in determining the intensity of
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12 emotional responses (McRae et al., 2008), the preferred emotion regulation strategies (Garnefski
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14 et al., 2004), and also the autonomic patterns displayed by individuals (i.e., HR/HRV profiles in
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16 response to different emotional scenarios; Koenig & Thayer, 2016; Smith et al., 2011). When
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18 under threat, previous studies have found that the attempt to suppress negative emotions could
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20 increase HRV (Butler et al., 2006; Smith et al., 2011), and that for females, a depletion in self-
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22 regulatory capacity could occur after facing negative stimuli (Smith et al., 2011). Additionally,
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24 the maturation of the ANS in adolescence seems to show different patterns depending on the sex
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26 of the individual (Harteveld et al., 2021).
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31 Even though the study of affect regulation in adolescence is of utmost relevance, there is no
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33 research addressing the physiological correlates of the three emotion regulation systems during
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35 this developmental stage and considering sex differences. One possible reason for this absence
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37 is that no study so far has produced/validated a set of standardized emotional scenarios that can
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39 be used to reliably elicit the three emotion regulation systems and explore their distinctive
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41 physiological patterns.
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44 To overcome these issues, two studies have been conducted and are presented in this paper.
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46 Study 1 aimed to develop a set of three emotional audio scenarios and validate a standardized
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48 procedure (SP), able to trigger the three systems independently. The SP was designed to be
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50 composed of resting, reactivity, and recovery phases for each system (Laborde et al., 2018).
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52 Study 2 aimed to explore different physiological and emotional correlates of the three emotional
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54 scenarios in adolescents. For the physiological measures, both HR and HRV indexes were
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56 explored. As primary indexes of PNS activity, the vmHRV indexes RMSSD and HFms² were
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1 considered (See measures section for detailed information on the vagally-mediated HRV
2 indexes).

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4 As regards the threat scenario, given its physiological basis (Mobbs et al., 2020; Porges,
5 2007; Richardson et al., 2016), decreases in vmHRV were expected from the resting phase to
6 reactivity, returning to resting levels in the recovery phase. Nonetheless, given the evolutionary
7 conception of the human mind and its threat focused nature (Gilbert, 2010), it is possible that in
8 the recovery phase, the stimulus will still continue to be processed, thus maintaining a decreased
9 PNS activity, reflected by decreased HRV. Moreover, previous research with adult samples has
10 pointed out that attempts to suppress negative emotions can increase HRV (Butler et al., 2006;
11 Smith et al., 2011) and that sex differences can be observed, with females displaying decreases
12 in resting HRV after facing stressful interactions, representing a depletion of self-regulatory
13 capacity (Smith et al., 2011). Thus, it is was hypothesized that the same pattern of results could
14 occur in the present study, when facing the threat scenario. For the emotional correlates,
15 negative affect was expected to increase in both sexes during the presentation of the scenario,
16 and to return to resting levels during recovery.

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18 For the drive system, since the experience of positive affect is associated with the action of
19 dopaminergic circuits and SNS activity (Richardson et al., 2016), decreases in HRV were
20 expected during reactivity when compared to the resting phase. Given the nature and function of
21 this system (Depue & Morrone-Strupinsky, 2005), HRV was expected to return to resting levels
22 at the recovery phase, showing an adaptive regulatory function (Laborde et al., 2018) since
23 positive mobilizing behavior is no longer needed after the drive stimuli. As regards the affective
24 correlates, activated affect (e.g., excitement, energy, and enthusiasm) was expected to increase
25 during the presentation of the drive scenario and to return to resting levels during the recovery
26 phase.

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28 During the presentation of the soothing scenario, since it is opiate/oxytocin mediated and
29 linked to PNS activity (Depue & Morrone-Strupinsky, 2005; Di Bello et al., 2020; McCorry,
30 2007), both HRV and the relaxed/safe positive affect were expected to increase from the resting
31 phase to reactivity. As regards the recovery phase, after increases in vmHRV in reactivity,

1 research points that the maintenance of increased HRV would indicate better self-regulation
 2 (Laborde et al., 2018). Nonetheless, these observations are based on challenging/demanding
 3 stimuli (Laborde et al., 2018) which is not the case of the soothing system. Additionally,
 4 following the evolutionary approach of the emotion regulation systems (Gilbert, 2010), soothing
 5 activity is associated with a general state of safeness, in which threat focus is inhibited as well
 6 as the pursuit of resources. Although HRV was expected to return to resting values, it is also
 7 possible that in the emotionally neutral recovery phase the soothing system would remain
 8 active, since mobilizing behavior (threat or drive) is not required. Thus, PNS activity,
 9 represented by vmHRV, could remain at reactivity levels in the recovery phase. No specific
 10 predictions were made for sex differences for the drive and soothing scenarios.

22 **2. Study 1 – Development and Validation of emotional scenarios**

23 Study 1 aimed to develop and validate standardized scenarios able to selectively trigger
 24 each emotion regulation system. Therefore, emotional scenarios were developed and validated
 25 by both experts and community adolescents. For both the resting and recovery phases,
 26 emotional neutral stimuli were selected.

33 **2.1 Method**

35 **2.1.1 Participants**

36 For the content validation of the initial scenarios, a convenience sample of 18 international
 37 experts in the affect regulation systems theory was contacted. Since educating patients about the
 38 three affect regulation systems (and their role in emotion regulation) is one of the core features
 39 of Compassion Focused Therapy (CFT) practice across different mental health conditions, at
 40 least 4 years of experience in the clinical application of CFT (Gilbert, 2010) were required.
 41 Thus, the final sample was composed of 14 experts with ages ranging from 33 to 66 years old
 42 ($M = 46.57$; $SD = 9.07$). The years of clinical experience in the application of the CFT model
 43 ranged from 4 to 20 years ($M = 8.50$; $SD = 4.75$). Although working across different fields,
 44 most of the experts (92.9%) reported the work as therapist/clinicians as their main professional
 45 activity.

1 For both the content and emotional validation of the scenarios a convenience sample of
2 community adolescents, composed of 31 participants (58.1% females; 41.9% males), was
3 recruited from Portuguese public schools. The age of the sample ranged between 14 and 18
4 years old (M = 15.71; SD = 1.27) and the completed years of education was on average 9.32
5 (SD = 1.38). There were no differences between sexes concerning Socio Economic Status
6 (SES¹) [$\chi^2(2) = .55$; $p = 0.759$], with 12.9% of the sample belonging to a low, 64.5% to a
7 medium, and 22.6% to a high SES. Also, there were no differences between sexes concerning
8 the years of completed education [$\chi^2(5) = .5.75$; $p = 0.331$].
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17 **2.1.2 Procedures**

18 Prior to data collection, this work was approved by a national ethics committee and by the
19 ethics committee of the Faculty of Psychology and Educational Sciences of the University of
20 Coimbra (the committee's approval was also extended to study 2). Informed consent was asked
21 from CFT experts before participating in the study. For the community adolescent sample,
22 school board's approval was required prior to data collection. Additionally, written informed
23 consent was asked from parents of students under 18 years old and from students with 18 years
24 old. Verbal consent was asked from all participants.
25

26 Initially, for each emotion regulation system, four written scenarios were developed by the
27 research team, describing adolescents' every-day life experiences considering the
28 developmental stage of the participants. Although scenarios were supposed to be emotionally
29 triggering, for ethical reasons, they were not designed to prompt strong emotional responses
30 (see Supplementary Material section for a brief description of each scenario).
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32 After the development of the twelve initial scenarios, experts in the affect regulation
33 systems theory were asked to rate them in terms of how representative of each system they
34 were. The presentation order of the scenarios was counterbalanced, controlling for potential
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36 ¹ Socioeconomic status (SES) was measured by parents' profession, considering the Portuguese
37 professions classification (Instituto Nacional de Estatística, 2010). Examples of professions in the high
38 SES group are judges, higher education professors, or MDs; in the medium SES group are nurses,
39 psychologists, or schoolteachers; and in the low SES group are farmers, cleaning staff, or
40 undifferentiated workers
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1 effects of a sequential rating procedure. This rating system allowed researchers to identify not
 2 only how well each scenario represented the targeted emotion regulation system but also how
 3 unrelated to the other systems the scenarios were. Based on these criteria, six scenarios were
 4 selected, two for each emotion regulation system.
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8 The scenarios were then presented to a sample of community adolescents and participants
 9 were asked to rate each scenario regarding their specificity to all emotion regulation systems.
 10 Participants were also asked to rate the intensity of their subjective emotional experiences for
 11 each scenario. Prior to these ratings, the researcher instructed all participants in the core features
 12 of the affect regulation systems theory.
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19 The standardized procedure contained resting and recovery periods, both with emotionally
 20 neutral content and with the length of exactly 5 minutes. Thus, a set of emotionally neutral
 21 pictures were selected from the International Affective Pictures System (IAPS; Lang et al.,
 22 1997; Soares et al., 2015). In total, 86 emotionally neutral images were selected from this
 23 database considering the ratings of the Portuguese validation (Soares et al., 2015). Each image
 24 was analyzed to assure that they were adequate to the developmental stage of the participants
 25 and that they were not related to the triggering stimuli.
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35 **2.1.3 Measures**

36 **Content appropriateness.** In order to validate the content appropriateness of each scenario
 37 (i.e., how accurately they represented each emotional system) a seven-point Likert scale
 38 (ranging from 1- *poor representation/ not related at all*; to 7 – *excellent representation/*
 39 *completely related*), was created. The rating of this scale allowed researchers to explore how
 40 well each scenario represented the targeted emotional system and how unrelated to the other two
 41 systems each scenario was.
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51 **State affect.** In order to validate the emotional content of each scenario (i.e., assess the type
 52 of affect elicited by each scenario), items describing different emotional states were selected.
 53 For the *threat system*, 5 items from the Positive and Negative Affect scale (PANAS; Watson et
 54 al., 1988) were selected and adapted (i.e., anger, shame, sadness, fear and anxiety). Items
 55 describing emotional states associated with the soothing and drive systems were selected and
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1 adapted from the Activation and Safe/Content Affect Scale (Gilbert et al., 2008). For the
2 *soothing system*, the selected emotional states were safeness, happiness, tranquility, calmness,
3 satisfaction and contentment. For the *drive system*, the selected emotional states were
4 excitement, energy, pleasure, vitality and enthusiasm. Participants rated each emotional state,
5 for each scenario, on a six-point Likert scale (ranging from 0 – not intense at all; to 5 –
6 extremely intense). Responses to these items were merged into three final scores. From now on,
7 the total score for the affect associated with the threat system will be called *negative affect*, for
8 the soothing system, the total score will be called *relaxed/safe positive affect* and for the drive
9 system, the total score will be called *activated positive affect*.

10 **International Affective Pictures System (IAPS; Lang et al., 1997; Portuguese version by**
11 **Soares et al., 2015).** The IAPS is an international database of affective stimuli used in research
12 on emotional and attentional processes. Stimuli are scored using three criteria (Lang et al.,
13 1997): 1) the emotional valence of the stimuli (positive or negative); 2) the perception of control
14 of the participants to the stimuli; 3) the degree of arousal that the stimuli prompts in the
15 participant. Based on these criteria (Soares et al., 2015), 86 emotionally neutral pictures were
16 selected for the resting and recovery phases.

17 **2.1.4 Data analysis**

18 The IBM SPSS Statistics v22 software was used to compute all data and carry out
19 statistical analyses. Descriptive statistics were performed to characterize the samples of experts
20 and community adolescents. In order to compare the ratings of the scenarios (both for content
21 and emotional validation), paired-samples T-tests were performed.

22 **2.2 Results**

23 **2.2.1 Experts ratings of the twelve initial scenarios**

24 For the content validation of the scenarios and to select two for each system, experts rated
25 the scenarios regarding their representativity of the three emotion regulation systems. Overall,
26 experts' scores for all scenarios showed that they were good representations of the systems they
27 were supposed to represent, given their higher scores in the targeted system when compared to
28 the scores of the remaining systems. All the explored differences within each scenario (e.g.,
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scores for the targeted system vs scores for the remaining systems) were significant ($p < .001$).

Thus, based on the experts' ratings, two scenarios were selected as being the best representation of each system. Mean scores of the experts' ratings for all scenarios as well as detailed description of the scenarios' selection process are presented in the Supplementary Material.

2.2.2 Content and emotional validation of the scenarios by community adolescents

To retain only one scenario for each emotion regulation system, community adolescents rated the six previously selected scenarios in terms of their representativity of all systems. Participants also rate the emotional states associated with each scenario. In order to retain only one scenario as the best representation of the targeted system, researchers considered the following criteria: higher representativity scores in the targeted system; lower representativity scores in the remaining systems; higher scores at the emotional states associated with the targeted system; lower scores at the emotional states associated with the remaining systems. For parsimony reasons, comparisons performed in the two scenarios representing each system are presented in the Supplementary Material.

Additionally, authors were also able to select which emotional states would be included in the final experimental procedure, based on two criteria: higher scores in the system they were related to; lower scores in the remaining systems. Therefore, for the threat system, the retained emotional states were anger, shame, sadness and fear. For the soothing system, safeness, calmness and tranquility were retained. Finally, for the drive system, excitement, energy and enthusiasm were retained.

For the final three scenarios, the emotional states associated with the targeted system were always significantly higher than the emotional states of the remaining systems ($p < .001$). For the threat system, the selected scenario refers to a situation in which the adolescent encounters his/her closest friends talking behind his/her back. A confrontation takes place and the adolescent ends up being excluded from the group. For the drive system, the selected scenario refers to a situation in which the adolescent must continuously save money to buy a pair of sneakers he/she really wants to possess. The scenario is being built up until the adolescent is finally able to buy them. Finally, for the soothing system, the scenario refers to a situation in

1 which the adolescent is holding a family baby that has fallen asleep. The baby awakes but with
2 some gentle, kind and warm words, the baby falls asleep again, in the arms of the adolescent.
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4 A brief description of the twelve initial scenarios is presented in the supplementary material
5 section.
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8 **3. Study 2 – Physiological and affective responses to the emotional scenarios**

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10 Study 2 aimed to first, build the final standardized procedure, and second, to explore the
11 physiological (HR/HRV) and emotional correlates of the three emotional scenarios (at resting,
12 reactivity and recovery), as well as to explore sex differences. To do so, prior to data collection,
13 a power analysis showed that a total sample of 179 community adolescents would be necessary
14 to detect medium effects with a significance level of 0.05 and a power of .80.
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22 **3.1 Method**

23 **3.1.1 Participants**

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25 In order to explore the psychophysiological correlates of the emotional scenarios, a total of
26 249 community adolescents were recruited from Portuguese Public Schools, aged between 14
27 and 18 years old. Participants composing the community adolescent sample for study 2 did not
28 overlap with participants recruited for study 1. To evaluate their eligibility for the study, the
29 following exclusion criteria was considered: 1) illiteracy; 2) obesity (body mass index (BMI)
30 greater than 30kg/m²); 3) psychopathology and/or presence of psychotic symptoms; 4) currently
31 under psychological treatment; 5) presence of cardiovascular diseases; 6) presence of
32 visual/hearing impairments or lack of glasses/hearing aid when needed; 7) intake of medication
33 or other illicit drugs with influence at the cardiovascular system.
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46 Of all participants contacted, 12 didn't fulfill the inclusion criteria (their age was not
47 included in the required age interval), 45 showed the presence of exclusion criteria and 27 were
48 excluded for not completing the data collection procedure. Finally, 10 participants were
49 excluded since they were identified as outliers in their physiological correlates. Thus, the final
50 sample was composed of 155 community adolescents. The mean age of the total sample was
51 16.17 (SD = 1.34) years old and composed of 70 males (45.2%) and 85 females (54.8%). There
52 were no differences between boys (M = 16.00; SD = 1.45) and girls (M = 16.31; SD = 1.24)
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regarding age ($t(153) = -1.42, p = .159$). The total sample had completed on average 10.12 (SD = 1.36) years of education and girls had significantly more years of completed education ($M = 10.35; SD = 1.26$) than boys ($M = 9.83; SD = 1.43; t(153) = 2.42, p = .017$). Of the total sample, 11.0% belonged to a low, 70.3% to a medium and 18.7% to a high socioeconomic status (SES²). There were no differences between sex regarding SES [$\chi^2(2) = 4.22; p = .121$]. Most of the sample didn't report visual problems (69%) and 31% had their eyesight problems corrected (e.g., glasses). There were no participants reporting hearing impairments. Finally, the average body mass index was 20.83 (SD = 2.19) and there were no differences between boys ($M = 20.81; SD = 2.25$) and girls ($M = 20.84; SD = 2.17; t(153) = .094, p = .925$).

3.1.2 Procedures

After the content and emotional validation of the scenarios (see Study 1; see Supplementary Material section), only three (one for each emotion regulation system) were selected and audio recorded in a controlled studio environment by a professional actor. Since the scenarios were developed to serve as triggering stimuli for the exploration of physiological response patterns (i.e., HR/HRV), they were edited to last exactly 5 minutes each, in line with the guidelines for short-term assessment of HRV (Laborde et al., 2017). In order to build the final version of the standardized procedure, the *Psychopy* program (Peirce, 2009) was used, allowing researchers to compute all the selected stimuli in a single experimental task (emotional scenarios and neutral images). The participants' degree of interaction with the experimental task was programmed prior to its execution. Therefore, participants were not able to change any aspect of the experimental task (e.g., timing, order or duration of the presented stimuli), assuring a standardized procedure.

Prior to data collection, school boards' approval was asked. The research goals were explained to participants and all ethical assurances were provided. Written informed consent

² Socioeconomic status (SES) was measured by parents' profession, considering the Portuguese professions classification (Instituto Nacional de Estatística, 2010). Examples of professions in the high SES group are judges, higher education professors, or MDs; in the medium SES group are nurses, psychologists, or schoolteachers; and in the low SES group are farmers, cleaning staff, or undifferentiated workers

1 was asked from both parents of students under 18 and participants with 18 years old. Verbal
2 consent was also asked from participants. Institutions were asked to signal adolescents between
3 14 and 18 years old with no history of disruptive behavior or other psychological issues. To
4 assure that none of the signaled adolescents had psychopathological symptoms, structured
5 clinical interviews (MINI-KID) were conducted with each participant. Being the first evaluation
6 moment, the clinical interview allowed for the exclusion criteria to be explored, assuring the
7 participants' eligibility to perform the standardized procedure.
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10 Participants were asked to restrain themselves, at least for two hours prior to the execution
11 of the SP, from extreme physical activity and from the consumption of caffeine or other
12 substances with influence at the autonomic level (i.e., medication and/or illicit drugs). In order
13 to minimize environmental interferences, the SP was performed in rooms with reduced noise
14 sources and always in the presence of the researcher.
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16 For the psychophysiological data measurement, the *Firstbeat Bodyguard 2* device
17 (Tarvainen et al., 2014) was employed. This noninvasive device allows for real time HR/HRV
18 measurement through two disposable electrodes placed on the participant's body. One is
19 attached to the right side of the body, bellow the collar bone. The other is attached to the left
20 side of the rib cage. Participants were asked to sit in front of the computer with their knees at a
21 90° angle, with both feet on the floor and hands on their thighs (Laborde et al., 2017). They
22 were then hooked up with soundproof headphones aiming to reduce external noise stimuli,
23 while being exposed to the SP. Additionally, all participants were instructed to remain as still as
24 possible during the SP, reducing potentially confounding cardiac activity in the recorded data.
25 ECG was continuously recorded thorough all the phases of the experiment.
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27 **3.1.3 Standardized Procedure for presenting emotional scenarios**

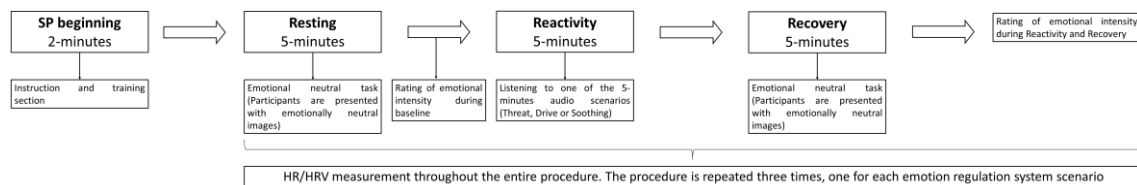
28 A standardized procedure for presenting emotional scenarios was built using the *Psychopy*
29 software (Peirce, 2009; see procedures section). It began with an instructions section, providing
30 participants with all relevant information for the correct execution of the experimental task.
31 During the first resting phase the selected emotional neutral stimuli were presented. Each image
32 from the IAPS lasted for 5s and they were randomly presented to participants (in each resting,
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as well as in each recovery phase, 60 images were presented). After the resting phase, participants answered the state affect scale regarding that specific phase (40s) in order to have an emotional baseline for all participants. Next, the first emotional scenario was presented to the participant in audio format, with the length of exactly 5 minutes. Immediately after, the recovery phase began. In this period, participants were exposed again to emotional neutral stimuli. After the recovery period, participants answered two state affect scales (with 40s each): the first referred to the recovery phase and the second referred to the emotional scenario. These scales were presented only after the reactivity and recovery phases, allowing for a continuous measurement of physiological data between them.

The presented sequence was repeated three times, one for each emotional scenario. The completed SP (see Figure 1) lasted for exactly 53 minutes. Since the SP contained resting, reactivity and recovery phases for each of the emotional scenarios, to reduce sequential interference, the presentation order of the systems was counterbalanced by creating six versions of the SP. Participants were randomly assigned to one of the six versions of the SP and there were no differences between sex regarding the frequency of these six versions [$\chi^2(5) = 2.051$; $p = 0.842$]. Moreover, preliminary multivariate analyses revealed no significant differences for males (Wilks' $\lambda = .957$, $F = .758$, $p = .641$, $\eta^2 = .022$) nor females (Wilks' $\lambda = .957$, $F = .914$, $p = .505$, $\eta^2 = .022$) in the physiological correlates of the three systems in their resting phases.

Figure 1

Schematic presentation of the standardized procedure



3.1.4 Measures

Clinical Interview

Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-KID; Sheehan et al. 2010; Portuguese Authorized Version by Rijo et al. 2016). The MINI-KID is a structured clinical interview that assesses DSM-5 diagnoses both in children and adolescents. All questions in the instrument are presented in a binary (i.e., yes/no) format. The MINI-KID is considered as being a short and accurate interview to diagnose a wide range of psychological disorders. It includes items to address medical, organic, and/or drug causes for disorders, also allowing the interviewer to decide which disorder should be consider the main diagnosis. The MINI-KID takes between 30 and 90 minutes on average to administer and inter-rater reliability was found to be excellent for all mental health disorders assessed (Sheehan et al. 2010).

Physiological measures

The ECG signal was digitized at 2000 Hz and inspected offline using Kubios software (Niskanen et al., 2004). Successive R waves (identified by an automatic beat detection algorithm) were visually inspected when suitable, a threshold-based artefact correction algorithm (very low: 0.45 seconds) was applied using the Kubios software. Heart Rate (number of heartbeats for time unit), and time and frequency domain indexes of HRV were obtained for each segment of interest of the experimental procedure (i.e., resting, reactivity and recovery phases for each emotion regulation system). In the time domain, the RMSSD (square root of the mean of the sum of the squares of differences between adjacent NN intervals) was computed (Laborde et al., 2017; Malik, 1996). In the frequency domain, following guidelines for reporting HRV indexes (Laborde et al., 2017; Malik, 1996) both the absolute power of the high-frequency band (0.15–0.4 Hz; HFms²) and the relative power of the high-frequency band in normalized units (HFu.u.) were computed. As the RMSSD and HFms² indexes are considered to be purer markers of PNS activity, the HFu.u. index is influenced by both PNS and SNS activity since it also accounts for the low frequency band (LF) of the ECG (Laborde et al., 2017; Heathers, 2014).

Analyses of normality were performed and following the guidelines of Laborde et al. (2017), when data was not normally distributed, logarithmic transformations were performed. Therefore, the RMSSD, HFms² and HFn.u were transformed with natural logarithms in all phases of the EP. Various physiological indexes were used given this is the first study directly assessing physiological correlates of the three emotion regulation systems in adolescents. Additionally, since the developed emotional scenarios were being used for the first time, we aimed to collect several PNS activity physiological indexes (HRV).

3.1.5 Data analysis

All data analyses were conducted with IBM SPSS Statistics v22. Preliminary analyses were conducted to identify outliers based on interquartile range rule provided by SPSS. To investigate for effects of the three different scenarios on the physiological measures and affect, a series of two-factor (i.e., between subjects— Sex— and within subjects—Time) mixed multivariate analyses of variance (MANOVA) were performed. Given the different nature of the constructs under assessment (physiological vs self-reported measures), for each emotional system, separate MANOVAS were performed. One with the physiological measures and other with the affect measures as dependent variables. Effect sizes for the Time and Time × Sex effects were computed using partial eta square (η_p^2), with $\eta_p^2 = .01$ referring to a small effect size, .06 to a medium effect size, and .14 to a large effect size (Tabachnick & Fidell, 2013).

3.2 Results

3.2.1 Physiological and emotional responses to emotional scenarios across sex

Threat System scenario

As regards the physiological responses to the threat system scenario, multivariate analyses revealed a significant effect of Time (Wilks' $\lambda = .916$, $F = 3.399$, $p = .001$, $\eta_p^2 = .043$), and a significant Time × Sex interaction (Wilks' $\lambda = .940$, $F = 2.364$, $p = .016$, $\eta_p^2 = .030$), both with small effect sizes. For Time, significant effects were found for the HFn.u. index, with a small effect size (see Table 2). Post hoc testing for HFn.u. revealed significantly higher scores at resting when compared to recovery ($p = .003$) and significantly higher scores during the threat scenario when compared to recovery ($p = .005$; see Table 1).

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2 As to the self-reported emotional responses, multivariate analyses revealed a significant
3 effect of Time (Wilks' $\lambda = .368$, $F = 65.624$, $p < .001$, $\eta_p^2 = .393$), with a large effect size.
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5 Although close to significance, there was no significant interaction between Time \times Sex (Wilks'
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7 $\lambda = .961$, $F = 2.042$, $p = .058$, $\eta_p^2 = .020$). Therefore, for Time, significant effects were found for
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9 the negative and relaxed/safe positive affect (with large effect sizes), and for the activated
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11 positive affect (with a medium effect size; see Table 2). Concerning negative affect, significant
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13 increases from both resting to the threat scenario ($p < .001$) and from resting to recovery ($p =$
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15 $.003$) were found. Also, from the threat scenario to recovery, a significant decrease was found (p
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17 $< .001$; see Table 1). As to the activated positive affect, significant decreases from resting to
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19 recovery ($p = .001$) and from the threat scenario to recovery ($p < .001$) were found. As regards
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21 relaxed/safe positive affect, a significant decrease from resting to the threat scenario ($p < .001$)
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23 and a significant increase from the threat scenario to recovery ($p < .001$), were found.
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25 Additionally, from resting to recovery, a significant decrease was also found ($p < .001$).
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29 Given that significant effects in Time \times Sex were found for the HFn.u. index (cf. Table 2),
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31 further univariate analyses were performed. Statistically significant differences were found for
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33 both male ($F = 6.398$; $p = .002$; $\eta_p^2 = .085$) and female adolescents ($F = 3.111$; $p = .047$; $\eta_p^2 =$
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35 $.036$) on HFn.u., with medium and small effect sizes, respectively. Post hoc testing revealed that
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37 male adolescents scored significantly higher at resting when compared to recovery ($p = .002$).
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39 Additionally, from resting to the threat scenario, a significant decrease was found for male
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41 adolescents ($p = .023$). For female adolescents, post hoc testing revealed a significant difference
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43 between the threat scenario and recovery ($p = .014$), with significantly higher scores during the
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45 threat scenario. No further differences were found for female adolescents. While male
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47 adolescents maintained a decreasing trend in this index across resting, reactivity and recovery,
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49 female adolescents seem to increase the HFn.u. index from resting to threat scenario, returning
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51 to resting scores in the recovery period.
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Table 1

Mean Scores and Standard deviations of the total sample in the physiological and emotional measures at resting, reactivity and recovery for the three Systems

	Threat scenario			Drive scenario			Soothing scenario		
	Resting	Reactivity	Recovery	Resting	Reactivity	Recovery	Resting	Reactivity	Recovery
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)
RMSSD	1.59 (.21)	1.59 (.21)	1.59 (.21)	1.59 (.22)	1.57 (.21)	1.59 (.21)	1.59 (.22)	1.60 (.22)	1.59 (.22)
HFms ²	2.80 (.44)	2.76 (.45)	2.77 (.45)	2.80 (.44)	2.73 (.44)	2.81 (.46)	2.81 (.46)	2.79 (.47)	2.79 (.46)
HFn.u.	1.55 (.25)	1.54 (.24)	1.51 (.23)	1.52 (.25)	1.53 (.23)	1.52 (.24)	1.55 (.23)	1.56 (.23)	1.50 (.25)
MeanHR	78.97 (10.75)	78.61 (10.48)	79.16 (10.47)	78.74 (10.62)	79.16 (10.41)	78.91(10.33)	78.30 (10.47)	77.60 (10.26)	79.08 (10.37)
Negative Affect	.36 (.61)	1.80 (1.24)	.48 (.74)	.33 (.54)	.27 (.50)	.30 (.54)	.36 (.61)	.26 (.50)	.33 (.57)
Activated PA	.98 (.95)	1.13 (.99)	.82 (.83)	.92 (.94)	2.55 (1.40)	1.01 (1.07)	.92 (.91)	1.30 (1.26)	.93 (.94)
Relaxed/Safe PA	2.89 (1.44)	1.01 (1.28)	2.57 (1.48)	2.85 (1.43)	2.13 (1.53)	2.84 (1.44)	2.82 (1.28)	3.68 (1.37)	2.97 (1.33)

Note. HRV = Heart Rate Variability; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; HFms² = absolute power of the high-frequency band; HFn.u. = relative power of the high-frequency band in normal units; MeanHR = mean heart rate; PA = Positive Affect; logarithmic transformations were performed in RMSSD, HFms² and HFn.u.

Table 2
 Mean Scores and Standard deviations for Both Sexes at resting, reactivity and recovery, and Univariate Tests during the Threat scenario

	Male Adolescents			Female Adolescents			Time	Time X Sex
	Resting	Reactivity	Recovery	Resting	Reactivity	Recovery		
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)		
RMSSD	1.62 (.20)	1.62 (.20)	1.62 (.21)	1.57 (.22)	1.56 (.22)	1.56 (.21)	F = .213; p = .808; $\eta_p^2 = .001$	F = 1.464; p = .233; $\eta_p^2 = .009$
HFms2	2.83 (.41)	2.81 (.44)	2.80 (.47)	2.77 (.46)	2.73 (.46)	2.75 (.44)	F = 1.613; p = .201; $\eta_p^2 = .010$	F = .228; p = .796; $\eta_p^2 = .001$
HFn.u.	1.52 (.24)	1.49 (.24)	1.45 (.24)	1.57 (.25)	1.59 (.22)	1.56 (.20)	F = 7.342; p = .001; $\eta_p^2 = .046$	F = 4.041; p = .019; $\eta_p^2 = .026$
MeanHR	76.40 (10.90)	75.89 (10.46)	76.78 (10.93)	81.09 (10.21)	80.85 (10.02)	81.12 (9.71)	F = 2.699; p = .069; $\eta_p^2 = .017$	F = .773; p = .463; $\eta_p^2 = .005$
Negative Affect	.35 (.64)	1.60 (1.23)	.51 (.72)	.36 (.58)	1.96 (1.23)	.46 (.76)	F = 194.210; p < .001; $\eta_p^2 = .559$	F = 3.943; p = .020; $\eta_p^2 = .025$
Activated PA	.93 (.96)	1.15 (1.03)	.73 (.79)	1.02 (.95)	1.12 (.97)	.90 (.87)	F = 10.543; p < .001; $\eta_p^2 = .064$	F = 1.021; p = .361; $\eta_p^2 = .007$
Relaxed/Safe PA	2.78 (1.55)	1.16 (1.39)	2.40 (1.50)	2.97 (1.34)	.89 (1.19)	2.71 (1.46)	F = 169.387; p < .001; $\eta_p^2 = .525$	F = 4.036; p = .019; $\eta_p^2 = .026$

Note. HRV = Heart Rate Variability; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; HFms² = absolute power of the high-frequency band; HFn.u. = relative power of the high-frequency band in normal units; MeanHR = mean heart rate; PA = Positive Affect; η_p^2 = partial eta square; logarithmic transformations were performed in RMSSD, HFms² and HFn.u.

Drive System scenario

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2 As regards the physiological responses to the drive system scenario multivariate analyses
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4 revealed significant effects for Time (Wilks' $\lambda = .922$, $F = 3.152$, $p = .002$, $\eta_p^2 = .042$), with a
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6 small effect size, but not for Time \times Sex (Wilks' $\lambda = .988$, $F = .460$, $p = .884$, $\eta_p^2 = .009$).
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8 Univariate testing revealed significant Time effects for RMSSD and HFms², with small and
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10 medium effect sizes respectively (see Table 3). Further post hoc testing revealed significant
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12 decreases from resting to the drive scenario ($p = .017$) and significant increases from the drive
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14 scenario to recovery ($p = .028$) in RMSSD index. Similarly, HFms² scores were higher at resting
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16 than during the drive scenario ($p < .001$) and increased from the drive scenario to recovery ($p <$
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18 $.001$; see Table 1).

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21 As to the self-reported emotional responses, significant effects were found for Time
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23 (Wilks' $\lambda = .460$, $F = 47.737$, $p < .001$, $\eta_p^2 = .322$), with a large effect size, but not for Time \times
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25 Sex (Wilks' $\lambda = .977$, $F = 1.167$, $p = .322$, $\eta_p^2 = .011$). Univariate testing revealed significant
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27 Time effects for activated and relaxed/safe positive affect, both with large effect sizes (see
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29 Table 3). Participants reported significantly higher activated positive affect during the drive
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31 scenario when compared to resting ($p < .001$). A significant decrease in the drive emotions was
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33 also found from the drive scenario to recovery ($p < .001$). There was no difference between
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35 resting and recovery ($p = .121$), showing that participants returned to resting levels of activated
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37 positive affect at recovery. As for relaxed/safe positive affect, post hoc testing revealed a
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39 significant decrease from resting to the drive scenario ($p < .001$) and a significant increase from
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41 the drive scenario to recovery ($p < .001$).

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Table 3

Mean Scores and Standard deviations for both Sexes at resting, reactivity and recovery, and Univariate Tests during the Drive System scenario

	Male Adolescents			Female Adolescents			Time	Time X Sex
	Resting	Reactivity	Recovery	Resting	Reactivity	Recovery		
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)		
RMSSD	1.62 (.21)	1.60 (.21)	1.62 (.22)	1.57 (.22)	1.56 (.21)	1.58 (.21)	F = 3.281; p = .039; $\eta_p^2 = .021$	F = .239; p = .788; $\eta_p^2 = .002$
HFms ²	2.84 (.43)	2.75 (.43)	2.82 (.47)	2.77 (.44)	2.72 (.45)	2.79 (.45)	F = 9.99; p < .001; $\eta_p^2 = .061$	F = .520; p = .595; $\eta_p^2 = .003$
HFn.u.	1.48 (.26)	1.47 (.23)	1.47 (.26)	1.56 (.24)	1.58 (.21)	1.56 (.22)	F = .103; p = .902; $\eta_p^2 = .001$	F = 1.035; p = .357; $\eta_p^2 = .007$
MeanHR	76.31 (10.78)	77.00 (10.61)	76.45 (10.55)	80.73 (10.12)	80.96 (9.95)	80.93 (9.74)	F = 1.517; p = .221; $\eta_p^2 = .010$	F = .531; p = .588; $\eta_p^2 = .003$
Negative Affect	.34 (.55)	.28 (.55)	.29 (.53)	.32 (.53)	.26 (.46)	.32 (.56)	F = 1.556; p = .215; $\eta_p^2 = .010$	F = .450; p = .618; $\eta_p^2 = .003$
Activated PA	.91 (.90)	2.33 (1.41)	.90 (.98)	.93 (.98)	2.71 (1.40)	1.13 (1.16)	F = 158.627; p < .001; $\eta_p^2 = .511$	F = 1.454; p = .235; $\eta_p^2 = .009$
Relaxed/Safe PA	2.69 (1.48)	2.07 (1.60)	2.63 (1.53)	2.98 (1.38)	2.17 (1.48)	3.01 (1.35)	F = 43.484; p < .001; $\eta_p^2 = .222$	F = 1.246; p = .289; $\eta_p^2 = .008$

Note. HRV = Heart Rate Variability; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; HFms² = absolute power of the high-frequency band; HFn.u. = relative power of the high-frequency band in normal units; MeanHR = mean heart rate; PA = Positive Affect; η_p^2 = partial eta square; logarithmic transformations were performed in RMSSD, HFms² and HFn.u.

Soothing System scenario

As regards the physiological responses to the soothing system scenario, multivariate analyses revealed significant effects for time (Wilks' $\lambda = .812$, $F = 8.336$, $p < .001$, $\eta_p^2 = .099$), with a medium effect size. No significant Time \times Sex interactions were found (Wilks' $\lambda = .983$, $F = .661$, $p = .726$, $\eta_p^2 = .009$). Univariate testing revealed significant Time effects on HFn.u. and HR (all with medium effect sizes; see Table 4). Post hoc tests revealed that HFn.u. scores were significantly higher at resting and during the soothing scenario than at recovery (both with $p < .001$). Although not statistically significant, reactivity scores for HFn.u. were higher than resting scores, following the expected pattern (see Table 1). For HR, resting scores were significantly higher than the scores during the soothing scenario ($p = .002$) and were significantly lower than the recovery scores ($p < .001$). Moreover, scores for HR during the soothing scenario were significantly smaller than recovery scores ($p < .001$).

As to the self-reported emotional responses, significant effects were found for Time (Wilks' $\lambda = .768$, $F = 14.296$, $p < .001$, $\eta_p^2 = .124$), with a medium effect size, but not for Time \times Sex (Wilks' $\lambda = .988$, $F = .619$, $p = .715$, $\eta_p^2 = .006$). Univariate testing revealed significant Time effects on all three types of affect (small effect size for negative affect, medium effect size for activated positive affect and large effect size for relaxed/safe positive affect; see Table 4). The relaxed/safe positive affect was higher during the soothing scenario than when compared to resting ($p < .001$). There was also a significant decrease of relaxed/safe positive affect from the soothing scenario to recovery ($p < .001$). As for threat focused negative affect, a significant decrease from resting to reactivity was found ($p = .028$). Finally, for the activated positive affect, a significant increase from resting to the soothing scenario and a significant decrease from the soothing scenario to recovery were found (both with $p < .001$).

Table 4
 Mean Scores and Standard deviations for Both Sexes at resting, reactivity and recovery, and Univariate Tests in the Soothing System

	Male Adolescents			Female Adolescents			Time	Time X Sex
	Resting	Reactivity	Recovery	Resting	Reactivity	Recovery		
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)		
RMSSD	1.62 (.20)	1.63 (.21)	1.64 (.21)	1.56 (.23)	1.57 (.23)	1.55 (.22)	F = .853; p = .427; $\eta_p^2 = .006$	F = 1.408; p = .246; $\eta_p^2 = .009$
HFms2	2.85 (.43)	2.83 (.47)	2.85 (.44)	2.78 (.48)	2.76 (.48)	2.74 (.47)	F = .570; p = .566; $\eta_p^2 = .004$	F = .869; p = .421; $\eta_p^2 = .006$
HFn.u.	1.52 (.23)	1.52 (.22)	1.46 (.28)	1.58 (.22)	1.60 (.23)	1.54 (.23)	F = 11.108; p < .001; $\eta_p^2 = .068$	F = .265; p = .768; $\eta_p^2 = .002$
MeanHR	75.75 (10.60)	75.33 (9.94)	76.42 (10.24)	80.40 (9.94)	79.47 (10.21)	81.27 (10.02)	F = 20.426; p < .001; $\eta_p^2 = .118$	F = 1.310; p = .271; $\eta_p^2 = .008$
Negative Affect	.36 (.61)	.28 (.50)	.38 (.63)	.36 (.62)	.25 (.50)	.29 (.52)	F = 3.553; p = .030; $\eta_p^2 = .023$	F = .920; p = .400; $\eta_p^2 = .006$
Activated PA	.80 (.83)	1.22 (1.30)	.89 (.92)	1.02 (.95)	1.36 (1.23)	.96 (.96)	F = 16.877; p < .001; $\eta_p^2 = .099$	F = .475; p = .623; $\eta_p^2 = .003$
Relaxed/Safe PA	2.73 (1.36)	3.53 (1.46)	2.92 (1.36)	2.89 (1.21)	3.80 (1.29)	3.02 (1.31)	F = 41.643; p < .001; $\eta_p^2 = .214$	F = .369; p = .691; $\eta_p^2 = .002$

Note. HRV = Heart Rate Variability; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; HFms² = absolute power of the high-frequency band; HFn.u. = relative power of the high-frequency band in normal units; MeanHR = mean heart rate; PA = Positive Affect; η_p^2 = partial eta square; logarithmic transformations were performed in RMSSD, HFms² and HFn.u.

4. Discussion

1 The affect regulation systems theory offers an evolutionary framework for the
2 conceptualization of emotion (dys)regulation, while proposing specific physiological correlates
3 for the three affect regulation systems (Gilbert, 2010, 2020; Richardson et al., 2016). In recent
4 years, research has been giving increased attention to HRV as a physiological correlate of
5 emotion regulation (Di Bello et al., 2020; Petrocchi et al., 2017; Thayer et al., 2012).
6 Specifically, increased HRV has been found to be associated with greater capacity for emotion
7 regulation (Thayer et al., 2012), greater capacity to engage in adaptive social relationships
8 (Porges, 2017) and increased compassion for the self and others (Di Bello et al., 2020).
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10 Nevertheless, until now, there was no study investigating HR/HRV patterns associated with
11 each specific emotion regulation system, during reactivity and/or recovery. The present research
12 aimed to fill this gap, by developing and validate specific emotional scenarios, and a
13 standardized presentation procedure which was able to sequentially trigger each specific
14 emotion regulation system in adolescents, allowing the investigation of HR/HRV patterns at
15 resting, reactivity, and recovery.
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17 In Study 1, the three emotional scenarios were developed and validated to represent the
18 three emotion regulation systems. The validation process of the emotional scenarios was based
19 on the ratings of CFT experts, as well as on the ratings of a sample of adolescents from the
20 community. Both the experts and the community adolescents recruited for study 1 rated each
21 final scenario as highly representative of the emotion regulation system it was intended to
22 trigger. Moreover, not only community adolescents rated the selected scenarios as being
23 strongly associated with the intended type of affect they were designed to trigger, but also, they
24 rated each final scenario as being poorly associated with untargeted types of affect. Thus, a
25 replicable standardized procedure for presenting these scenarios was built and used in Study 2.
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27 Study 2 aimed to investigate differences in HR/HRV specifically associated with each
28 emotion regulation system. Both time-domain (i.e., RMSSD) and frequency-domain measures
29 (i.e., High Frequency measures) were analyzed. Although sometimes challenging to interpret,
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1 this is a recommended research practice when assessing vagally-mediated HRV (Laborde et al.,
2 2017), allowing for a broader examination of HRV indexes and their relation to each other.
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4 For clarity reasons, findings will be discussed for each specific emotion regulation system at
5 a time, firstly addressing physiological data and the associated self-reported emotional states for
6 the total sample, followed by the discussion on sex differences.
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10 The activation of the threat system is linked to negative affect, decreased executive
11 functioning (Gilbert, 2010, 2019), greater SNS activity, increased HR and decreased HRV
12 (Richardson et al., 2016). In the present research, as regards the threat system scenario,
13 significant effects across the different phases of the SP were found for the HFn.u. index. This
14 HF-HRV measure presented a decreasing trend from resting to recovery. It should be noted
15 however that this index is not considered a pure marker of PNS activity since it accounts for
16 both the HF and low frequency (LF) bands of the ECG. Nonetheless, the physiological data for
17 the total sample showed that participants' HFn.u. values did not return to resting levels in the
18 recovery phase. The arithmetic relation between HFn.u. and LFn.u. ($LFn.u. = 1 - HFn.u.$;
19 Heathers, 2014) allow researchers to identify both indexes values and patterns. Moreover,
20 fluctuations in the LF band are thought to be dominated by SNS activity (Furlan et al., 2000;
21 Malik, 1996). Although no significant differences were found in purer PNS indexes (i.e.,
22 RMSSD and HFms²), since HFn.u. decreased from resting to recovery, it is possible that
23 increases in the LF band also occurred which could be associated with SNS functioning. In fact,
24 when facing socially threatening situations, individuals are expected to increase both cortisol
25 levels (Steimer, 2002) and SNS activity in the cardiac output, causing HRV to decrease and
26 preparing the organism for fight and flight responses (Dickerson & Kemeny, 2004; Porges,
27 2007).
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51 Regarding the threat focused negative affect, participants significantly increased both from
52 resting to reactivity and from resting to recovery. Although negative affect decreased from
53 reactivity to recovery, it did not return to resting levels. These findings are in line with the
54 physiological findings, indicating that participants did not return to basal levels in the recovery
55 phase, suggesting a slow recovery from a mild threat. It is possible that participants maintained
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cognitive processing, experiencing spontaneous recall, intrusion, or rumination of the stimuli even during the recovery phase.

During the activation of the threat system, drive related emotions significantly decreased from resting to recovery and from the reactivity to the recovery phase. These findings support the view that threat processing is accompanied by decreases in positive emotional states, such as those linked to the drive system (e.g., excitement, enthusiasm). This makes evolutionary sense because individuals need to attend to the threat rather than resource seeking and so those emotions and concerns are turned off. Finally, emotions associated with the soothing system (e.g., safeness, calmness, tranquility), significantly decreased from resting to reactivity of the threat system. Taken together, these findings offer support for the theoretical assumption that the triggering of the threat system is associated with decreased PNS activity, decreases in HF-HRV indexes, and the inhibition of both drive and soothing related emotions.

When analyzing HR/HRV patterns related to the threat system scenario across sex, differences were also found for HFn.u. index. Male adolescents displayed a decreasing trend in HRV, from resting to recovery. As for females, no differences were found in the HFn.u. between resting and reactivity (although HRV was higher in the reactivity phase). Nonetheless, the decrease in HRV between reactivity and recovery was significant for females. Greater prefrontal cortex activity has been found to be associated with increased HRV (Thayer et al., 2009), and our findings are in line with McRae and colleagues (2008) study, where males showed lesser increases in prefrontal activity when facing negative stimuli, when compared to females. Also, previous research with adults, pointed out that attempts to suppress negative emotions in social interactions could increase HF-HRV (Butler et al., 2006; Smith et al., 2011) and that after stressful interactions, decreases in resting HF-HRV in females could occur, representing a depletion of self-regulation capacity (Smith et al., 2011). Additionally, previous research has also pointed out that sustained attention to maintain social behavior is accompanied by smaller cardiac vagal control decrease (Beauchaine et al., 2007) and that successful regulation of emotion during emotion regulation tasks is associated with cardiac vagal control increase (Park et al., 2014). Another possible explanation for these findings refers to sex

1 differences in the maturation of the ANS during adolescence. The ANS maturation seems to
2 occur earlier for female adolescents when compared to males (Harteveld et al., 2021).

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4 Moreover, although SNS activity decreases from childhood to late adolescence in both sexes,
5 the PNS seems to mature earlier in females (Harteveld et al., 2021). Thus, it is possible that
6 female adolescents have increased PNS resources available, being more able to inhibit SNS
7 activity when confronted with a mild threat.
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13 Regarding the different types of affect elicited in the threat scenario, no sex differences
14 were found. Although previous research pointed out that boys seem to have more intense
15 emotional responses and take longer to recover from threat stimuli, especially when
16 experiencing emotions such as anger (McRae et al., 2008), in the present study, these
17 differences did not emerge. Even if no differences were found between sex, for the different
18 types of affect elicited by the threat scenario, differences were found for the physiological
19 correlates. This finding should be considered in future studies exploring the affect regulation
20 systems theory. Although self-reported affect might not differ between sex, the underlying
21 physiological patterns might play a relevant role in the selection of adaptive emotion regulation
22 strategies. Overall, findings related to the type of affect associated with the threat scenario are in
23 line with theory, stating that, when under threat, individuals are expected to reduce the ability to
24 display socially adaptive interactions as well as affiliative emotions (Gilbert, 2019; Porges,
25 2017).
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42 The drive system associates with dopaminergic circuits and SNS activity, increasing HR
43 and decreasing HRV (Richardson et al., 2016). In the present research, when analyzing data
44 related with the drive system scenario, both the RMSSD and HFms² indexes significantly
45 decreased from resting to reactivity, returning to resting levels in the recovery phase. These
46 findings agree with previous research concerning the action of the SNS and dopaminergic
47 circuits in positive mobilizing behavior, causing HRV to decrease (Richardson et al., 2016).
48 Moreover, the decrease in vmHRV indexes was accompanied by an increase in drive related
49 emotional states, which also returned to resting levels during the recovery phase. Threat related
50 emotions showed no differences across the different phases of the SP when triggering the drive
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system, which may be explained by the lower ratings of these emotions at resting.

Contrastingly, soothing related emotional states showed a decrease during the triggering of the drive system. A possible explanation for these findings is that SNS activity, with decreased vmHRV, in the activation of the drive system, was able to inhibit PNS activity in the heart and reduce affiliative emotions associated with the soothing system (e.g., safeness; calmness; Gilbert, 2019). No sex differences in HR/HRV patterns were found when triggering the drive system. The similarity of the physiological and emotional patterns between sexes might indicate not only that the drive scenario was able to trigger the targeted system, but also that the physiological mechanisms underlying the activation of this system are similar between male and female adolescents.

The soothing system is linked to affiliation and prosocial behavior, and, at the physiological level, involves increased PNS activity, decreased HR and increased HRV (Di Bello et al., 2020; Gilbert, 2010). This system plays a major role in regulating both the drive and the threat systems (Armstrong III et al, 2020; Gilbert, 2010). When considering the activation of the soothing system, differences across the different phases of the SP were found for the HFn.u. index in the present research. This HF-HRV index decreased from resting to recovery and between reactivity and the recovery phase, but no significant differences were found between resting and reactivity. In contrast with the HRV indexes, HR decreased from resting to reactivity and it increased from reactivity to recovery. Also, in the recovery phase, HR was significantly higher than in the resting phase. These findings offer preliminary evidence for the view that the soothing system is linked to increased PNS and reduced SNS activity (Di Bello et al., 2020; Gilbert, 2014, 2020). Working as a toning-down strategy for both the threat and drive systems, it is possible that the soothing system may work as the “background” system and that it represents the “being mode” in psychologically healthy individuals (Gilbert, 2014). This assumption could also explain why differences between resting and reactivity were not significant when participants were exposed to a mild soothing stimulus. In view of these physiological data, it is possible that after the triggering of the soothing system, a depletion of

1 PNS activity might have occurred, explaining the lower HRV and higher HR in the recovery
2 phase, when compared to resting and reactivity.
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4 Soothing related emotional states showed a pattern congruent with physiological findings.
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6 Soothing related emotions increased from resting to reactivity and returned to resting levels in
7 the recovery phase. At resting, these emotional states were higher than the emotional states
8 associated with the other two systems, potentially explaining a less expressive increase in HRV
9 indexes from resting to reactivity. Regarding negative affect in the activation of the soothing
10 system, threat related emotions decreased when triggering a relaxed/safe type of affect,
11 reinforcing the capability of the soothing system to tone down threat related emotions.
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13 Interestingly, drive related emotions also increased from resting to reactivity. Although not so
14 expressive as the increases in the soothing related emotional states, these findings are not
15 surprising, given the positive valence of both the soothing and drive emotions. Therefore, it is
16 possible that a mobilizing and enthusiastic interpretation of the soothing scenario might have
17 occurred for some participants, diminishing the activity of the PNS in the cardiac output during
18 the reactivity phase. No sex differences were found during the soothing system SP, neither at a
19 physiological level, nor when analyzing emotional states, although previous studies have
20 pointed out possible hormonal differences between sexes that could influence PNS activity
21 (Koenig & Thayer, 2016).
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40 Overall, findings are in line with the affect regulation systems theory assumptions,
41 concerning system specific physiological and emotional patterns. Both physiological and
42 emotional findings support that each system can be independently triggered with mild stimuli.
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44 The emotional states associated with each specific affect regulation system should be considered
45 when exploring physiological correlates of the threat, drive and soothing systems. Additionally,
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47 research has been reporting decreased HRV as a general marker of decreased capacity for
48 emotion regulation (Porges, 2017; Thayer et al., 2012). However, in the present research, for the
49 triggering of both the threat and drive systems, reduced PNS activity with reduced HRV was
50 found. The interpretation of decreased HRV as an indicator of diminished capacity for the
51 implementation of adaptive emotion regulation strategies should also be cautious. It is possible
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1 that decreased PNS activity and HRV, in the activation of the drive system, may represent
2 adaptive physiological patterns, allowing the organism to pursue and accomplish important
3 resources and goals. The organism is dynamic and should be flexible when adapting to
4 environment demands. Similarly, HR/HRV should also be flexible, not static. Therefore, future
5 explorations of physiological correlates of emotion regulation should address not only
6 participants' emotional states, but also pay attention to the context where physiological indexes
7 are collected. This study adds to previous knowledge on the assessment of physiological
8 functioning in different contexts/scenarios and, in doing so, provides ecological validity to
9 findings.

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11 This research is not free of limitations and future research should try to overcome them.
12 Respiration control was not assessed. Nonetheless, the HRV indexes used are thought to be
13 robust enough towards the respiratory influence, specially the RMSSD index (Laborde et al.,
14 2017). For female adolescents, the phase of the menstrual cycle, as well as pill intake, were not
15 assessed. In addition to these limitations, future studies should also explore HR/HRV patterns
16 across the three emotion regulation systems, using different and stronger stimuli, allowing for
17 further investigation of the specific physiological patterns associated with each system.
18 Moreover, it was unclear why, across the activation of the different systems, differences in
19 physiological data were located in different HRV indexes. The exploration of this issue might
20 help to clarify this research field and points out the need for researchers to report various HRV
21 indexes. Also, future research should use physiological measures that directly assess SNS
22 activity. Finally, future studies using this same standardized procedure should resort to larger
23 samples and relate the physiological data with relevant self-report data such as difficulties in
24 emotion regulation strategies, since this information might clarify the relation between
25 physiological and emotional correlates of the three systems. Since adolescents are more prone to
26 emotion dysregulation, to display higher frequency of risk behaviors (Desatnik et al., 2017;
27 Theurel & Gentaz, 2018) and to present an increased risk for the development of
28 psychopathology (including internalizing and externalizing disorders; McLaughlin et al., 2011),
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1 the SP used in the present research can be used to explore differences in autonomic activity in
2 at-risk and/or clinical samples.
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4 Globally, our findings offer new evidence supporting the affect regulation systems theory
5 assumptions, namely the existence of three distinct emotion regulation systems, linked to
6 specific physiological and emotional patterns. The present study is the first to explore HRV
7 patterns in community adolescents through a standardized experimental procedure, specifically
8 designed to trigger each system independently. In doing so, different parasympathetically
9 mediated HRV indexes were explored, showing concurrent results across the physiological
10 measures and self-reported emotional states, thus corroborating the use of HRV as a
11 physiological correlate of emotion regulation.
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