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BRIEF REPORT

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# Covariate effects of resting heart rate variability on affective ratings and startle reflex during cognitive reappraisal of negative emotions

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#### ABSTRACT

Heart Rate Variability (HRV) has been widely studied in laboratory settings, primarily due to its clinical implications as a potential biomarker of emotion regulation (ER). Studies have reported that individuals with higher resting HRV show more distinct startle reflex responses to negative stimuli as compared to those with lower resting HRV. These responses have been associated with better defense system function when managing the demands of the context. There is, however, a lack of empirical evidence on the association between resting HRV and startle reflex during laboratory tasks using instructed ER. This study aims to explore the influence of resting HRV on voluntary cognitive reappraisal through subjective and startle reflex responses measured during an independent emotion regulation task. In total, 122 healthy participants completed a task consisting of attempts to upregulate, downregulate, or react naturally (non-regulate) to emotions prompted by unpleasant pictures. Tonic HRV was measured for 5 min before the experiment began. The results of the current study did not support the idea that self-reported and startle reflex responses were influenced by resting HRV. These findings suggest that, irrespective of resting HRV, individuals may benefit from strategies such as cognitive reappraisal that are useful for managing negative emotions. Experimental studies should further explore the role of individual differences in the use of ER strategies during laboratory tasks.

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#### KEYWORDS

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rate variability; startle reflex	70

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One of the most widely researched emotion regulation (ER) strategies is cognitive reappraisal, an antecedent-focused strategy that is implemented before an emotional response is prompted (Gross & John, 2003). This strategy can be used to voluntarily increase or decrease emotions through the modification of one's thoughts about a situation and, therefore, its emotional impact. The various affective, cognitive, and social benefits of using reappraisal have consistently been reported in the literature (i.e.
 Szasz et al., 2011). Similarly, poor cognitive reappraisal implementation has been considered to be a core

feature of several emotional disorders, including anxiety and depression (Garnefski & Kraaij, 2018).

Different theories have emerged to explain the neural basis of self-regulatory emotion processes. One of the most influential theories is the Neurovisceral Integration Model (Thayer & Lane, 2000). This theoretical model emphasises the relevance of negative feedback and inhibitory processes of the central nervous system in cognitive, affective, and autonomic regulation. Specifically, the Neurovisceral Integration Model focuses on central autonomic network (CAN) function, an integrated component of an internal

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<sup>90</sup> 

regulation system through which the brain controls

the visceromotor, neuroendocrine, and behavioural

responses essential for survival (Benarroch, 1993). In

this sense, the CAN is a critical functional network

for goal-directed behaviour and adaptability in chan-

ging environments. The network is composed of cor-

tical and subcortical areas such as the anterior

cingulate, insular and ventromedial prefrontal cor-

tices, and the central nucleus of the amygdala,

among others (Benarroch, 1993). The primary output

of the CAN is mediated via sympathetic and parasym-

pathetic neurons, which innervate the heart through-

information from peripheral organs such as the

heart is fed back to the CAN. The output of this func-

tional network appears to be directly linked to heart

rate variability (HRV), and is considered by many

researchers to be an index of the efficiency of

central-peripheral neural feedback mechanisms and

central nervous system-autonomic nervous system

integration (Thayer & Lane, 2000). Accordingly, HRV

could be involved in self-regulatory processes and

efforts to adapt to environmental demands, for

example in the implementation of cognitive reapprai-

sal (Thayer & Lane, 2000, 2009). In this regard, higher

HRV in resting states has been related to greater pre-

frontal-amygdala pathway functional activity, which

is associated with better cognitive ER (Wei et al.,

2018). Indeed, people with high HRV show improved

psychological flexibility and less psychopathology

(Beauchaine & Thayer, 2015). Likewise, a recent

study suggested that individuals with low HRV could

have difficulties in the activation of prefrontal struc-

tures, which are implicated in the modulation of

amygdala activity during cognitive reappraisal (Stein-

sensory

the vagus nerve. Furthermore,

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furth et al., 2018). Another peripheral measure that has been closely linked to amygdala activity is the startle reflex (Angrilli et al., 1996; Davidson, 2000; Kuhn et al., 2020; Lang et al., 1992). This is an index of defensive 140 response that can be measured through the EMG activity of the orbicularis oculi (Bradley et al., 1999; Lang, 1995). The startle reflex has proved to be an effective correlate of emotion reactivity and regulation processes (Lang et al., 1990; Speed, 2012; 145 Zaehringer et al., 2018). Specifically, eyeblink amplitude can be modulated depending on the affective content of the foreground, as it is potentiated during the presentation of negative stimuli and inhibited in the case of positive stimuli (Bradley 150 et al., 1991).

during cognitive reappraisal, the literature has revealed important discrepancies, particularly in relation to the downregulation of negative emotions (Zaehringer et al., 2020). On the one hand, some 155 studies have reported larger eyeblink amplitudes when participants increase their negative emotions and smaller blinks when negative emotions are decreased (Bernat et al., 2011; Conzelmann et al., 2015; Jackson et al., 2000). By contrast, other investi-160 gations have failed to replicate meaningful changes in blink amplitude in association with downregulation (Eippert et al., 2007; Fuentes-Sánchez et al., 2019; Ray et al., 2010). These divergences could be explained by the remarkable methodological discrepancies across 165 the studies, such as the inconsistency in cues and emotion regulation instructions, among others (Bernat et al., 2011; Fuentes-Sánchez et al., 2019). They could also be attributed to the role of individual differences in cognitive reappraisal, which could 170 modulate voluntary regulation outcomes (Mauss et al., 2007). In this line, differing effects of instructed reappraisal have been found in tense social interactions, and reappraisal instructions were only found to be effective for high habitual reappraisers who 175 exhibited lower cardiovascular and cortisol reactivity (Mauersberger et al., 2018). However, little is known about the effects of physiological biomarkers such as HRV. Specifically, it is unclear whether they should be considered as covariates that can 180 influence cognitive reappraisal.

Regarding the modulation of the startle response

In this regard, research among diverse paradigms has shown that people with high HRV show more distinct startle reflex responses (Sevenster et al., 2015). More specifically, this is evidenced by enhanced 185 startle reflex responses to unpleasant experiences and reduced blink responses to pleasant content as compared to neutral foregrounds (Bos et al., 2013; Ruiz-Padial et al., 2003; Ruiz-Padial & Thayer, 2014). This pattern could be explained by appropriate 190 defense system activation in response to the demands of the context. In this regard, the startle response reflects the potentiation of subcortical structures (such as the amygdala) in negative contexts, whereas an inhibition of such structures occurs 195 during positive stimuli. Moreover, individuals with low HRV appear to show undifferentiated startle reflex responses when faced with neutral and unpleasant pictures, suggesting difficulties in recognising safety signals (Ruiz-Padial & Thayer, 2014). Similarly, 200 Wendt et al. (2015) reported that individuals with

low HRV showed a greater difficulty in applying previously learned safety signals and acquiring new safety knowledge under the threat of shock. Furthermore, higher levels of HRV have been related to increased safety learning (Pappens et al., 2014). In addition, Melzig et al. (2009) investigated the relationship between HRV and startle reflex using a threat-ofshock paradigm, reporting that lower HRV was associated with greater startle reflex responses during periods of threat but not safety. This suggests that HRV is associated with fear-potentiated startle and not with the startle reflex per se.

Despite these findings, there is a lack of empirical evidence on the association between resting HRV and the startle reflex during experimental tasks in 215 which ER strategies such as cognitive reappraisal are implemented. In this study, we examined the effect pattern of cognitive reappraisal with regard to subjective and startle reflex responses to unpleasant pictures, while also considering resting HRV scores. 220 Specifically, participants were instructed to voluntarily down- or upregulate their negative emotions. To measure the effectiveness of ER, we included two affective ratings (valence and arousal), as well as startle reflex modulation. We hypothesised that participants with higher HRV would show reduced subjective ratings and eyeblink magnitude as compared to those with lower HRV when instructed to downregulate their negative emotions. Conversely, we expected to find increased reactivity in those individ-230 uals with higher HRV when instructed to increase their emotions in response to unpleasant pictures.

#### Materials and methods 235

#### **Participants**

This secondary research study was derived from a larger project (Fuentes-Sánchez et al., 2019) con-240 ducted to explore the relationship between HRV and self-report and startle reflex measures. The original study sample comprised 122 participants, including undergraduate students (40%) from Jaume I University (Spain) and healthy volunteers (60%). In this study, 11 participants were removed due to cardiac 245 problems (n = 1), medication use (n = 3), or failures in ECG data collection (n = 7). Furthermore, 8 participants were excluded due to additional failures in subjective ratings, and 11 due to high non-response rates with regard to the startle reflex (overall non-response 250 within individuals of over 65% in trials) or failures in

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startle reflex recording. Therefore, 103 participants were included in the statistical analysis of affective ratings (mean age = 24.94; SD age = 4.05; 62.1%female) and 100 in the analysis of startle reflex (mean age = 24.84; SD age = 3.99; 62% female). Ethical approval from the Deontological Commission at Universitat Jaume I was obtained, and the study was conducted in accordance with the Declaration of Helsinki.

#### Stimuli and design

In total, 70 pictures (60 unpleasant and 10 neutral) were selected from the International Affective Picture System (Lang et al., 2008) based on the Spanish normative values for affective valence and arousal (Moltó et al., 2013).<sup>1</sup> For each unpleasant condition (up-regulation, down-regulation, look), 20 unpleasant pictures were presented. In addition, 10 neutral pictures were included solely for the nonregulation or control condition (look). These stimuli were distributed into two series which consisted of five blocks with seven pictures each (6 unpleasant, 1 neutral). The normative affective ratings for each category were as follows: unpleasant pictures (valence: M = 2.14, SD = 0.44; arousal: M = 6.87, SD = 0.49); neutral pictures (valence: M = 5.09, SD = 0.28; arousal: M = 2.69; SD = 0.41).

Each trial began with a cue word ("up", "down", or "look") presented in the centre of a black screen for 280 2 s. Then, an unpleasant or neutral picture was presented for 8 s, and participants followed the cued instruction to either "look" or regulate their emotions. Specifically, when the cue was "look", participants were instructed to show a natural response to the 285 upcoming scene. Likewise, when the "up" cue was shown, the participants enhanced their feelings toward the forthcoming picture. To do so, they could implement different cognitive strategies such as thinking that "this is real", "things will get worse 290 over time", or "things are even worse than they appear to me". In the case of the "down" cue, parti cipants were instructed to reduce their feelings with regard to the upcoming picture. To this end, they could use cognitive strategies such as thinking that "this is not real", "things will improve with time", or "things aren't as bad as they appear to me" (McRae et al., 2008). The participants then reported their affective responses to the pictures using the Self-Assessment Manikin (SAM; Lang, 1980), a non-verbal pictorial assessment technique with a nine-point

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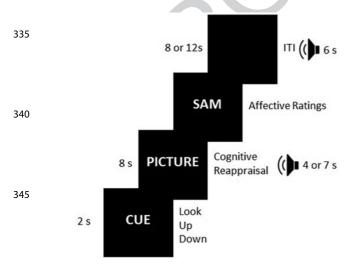
scale for measuring valence (pleasant-unpleasant) and arousal (calm-aroused). Participants were not given a specific time limit to rate each picture, but the computer screen automatically switched to the next image after each affective rating was provided using the keypad. Each trial ended with an intertrial interval (ITI) ranging from 8 to 12 s (see Figure 1).

In order to prompt eyeblink responses, acoustic probes with digitised white noise (50 ms, 105 dB) were binaurally presented over Sennheiser HD-205 headphones either 4 or 7 s after picture onset. In total, 72 probes were utilised (2 during training trials, 56 during picture viewing, and 14 during ITIs of 12 s).

Physiological recording and data reduction

All raw psychophysiological signals were recorded using a Biopac MP36R four-channel data acquisition and analysis system (Inc., Goleta, CA, USA).

The electrocardiogram was recorded using AG/ AgCl electrodes placed in a lead-II configuration. Interbeat intervals (IBIs) were measured as the time interval between consecutive R waves (1000 Hz, 0.5-35 bandpass filter). IBIs were extracted offline using Acqknowledge 4.2 software. These were visually inspected, and correction of artifacts in RR intervals was performed by hand. Resting HRV was obtained offline from the 5-min recording before the ER task through the Root Mean Square Successive Differences (RMSSD) of the interbeat interval time series obtained



350 Figure 1. Schematic representation of the trial structure in the emotion regulation task.

using Kubios 3.0.2 software (Tarvainen et al., 2014). The RMSSD reflects cardiac parasympathetic influences on the heart and is commonly understood to be a vagal-mediated HRV index (Malik et al., 1996). The absolute values of RMSSD were transformed logarithmically in order to normalize the distribution (see raw and transformed HRV data in Table 1). <sup>2</sup>The results of the high-frequency component of HRV (HF-HRV: 0.15–0.4 Hz) are also reported in Supplemental Material 1.

The startle reflex was recorded electromyographically through the orbicularis oculi muscle using two 4-mm Ag/AgCl electrodes placed below the left eye. The raw signal was sampled at 1000 Hz and filtered online with a high-pass (30 Hz) and low-pass 365 (500 Hz) filter. This signal was integrated and rectified online using Root Mean Square (RMS) integration with a time constant of 20 ms. Blink responses were visually inspected, with peaks detected using Acqknowledge 4.2 software. Eyeblink magnitude 370 was defined as the difference between baseline (average over 20 ms before probe onset) and peak (from 21 to 160 ms after probe onset). Trials in which eyeblinks were outside this range or could not be discerned from surrounding noise were 375 classified as missing in the posterior statistical analyses. Raw values were standardised (separately for each participant) on the basis of the mean and standard deviation of blinks elicited during ITIs. Blinks were expressed as T-scores ([z \* 10] + 50). In this stan-380 dardisation technique, a T-score of 50 indicates reflexes identical to those elicited during the ITI, and experimental blinks are not in the same distribution as the reference (ITI), providing independent standardisation (Bradford et al., 2015). 385

#### Procedure

Upon arrival at the laboratory the participants read an overview of the task and completed a written informed consent form. They were then asked to sit in an armchair in a dimly lit room, and sensors were attached. HRV was subsequently recorded for 5 min. Participants were then instructed with regard to the

Table 1. Raw and logarithmically	transformed values for RMSSD.
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	Interbeat interval (RR) (msec)		
	Mean	SD	Range
RMSSD (ms)	34.82	18.52	7.61–105.26
logRMSSD (ms)	3.42	.51	2.03-4.66

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trial structure, the ER task, and the rating of their affective responses. Before the task began, practice trials were performed to verify that participants understood the procedure. Afterwards, the experimental task was performed over a period of approximately 30 min.

#### Data analyses

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For all measures, averages for the downregulation, look, and upregulation conditions were calculated. In order to explore emotional reactivity, preliminary analyses were performed by means of separate ANCOVA measures, with the picture category (unplea-415 sant, neutral) as the within-subjects factor and HRV as the covariable. Only trials belonging to the look condition were included in this data analysis. To explore whether the HRV moderated the relationship between reappraisal and startle reflex modulation, a 420 Cue x HRV ANCOVA was performed separately for each dependent measure. For this, the cue ("up", "look", or "down") was set as the within-subjects factor, and HRV was included as a covariable. HRV was centred prior to ANCOVA analyses because the 425 data contained within-subject factors (Schneider et al., 2015). Assumptions of the normality, homoscedasticity, sphericity, and equality of variances were explored using the Mauchly test and Greenhouse-Geisser correction, where appropriate. Post-hoc t-430 test comparisons were performed to explore plausible differences between pairs of cue conditions when suggested by the results of the repeated-measures ANCOVA. Partial eta squared ( $\eta_p^{p, 22}$ ) and Cohen's d are reported as measures of effect size. All statistical 435 tests were conducted using SPSS statistical package version 23.

Table 2. Means (± SD) and confidence intervals (CI) for affective
 ratings and startle reflex during emotion reactivity separately for
 each picture category.

	····   ······			
		Overall		
			95% CI	
		Mean (SD)	Lower	Upper
445	Valence ratings			
445	Neutral	5.14 (0.67)	5.01	5.27
	Unpleasant	2.60 (0.86)	2.43	2.77
	Arousal ratings			
	Neutral	1.66 (1.09)	1.44	1.88
	Unpleasant	5.75 (1.56)	5.44	6.05
	Startle reflex			
450	Neutral	48.69(3.84)	47.93	49.46
	Unpleasant	49.97 (3.54)	49.28	50.66

#### Results

#### Resting HRV and emotion reactivity

The picture category was primarily found to influence affective valence (F(1, 101) = 427.33, p < 0.0001,  $\eta_p^2 = 0.809$ ), emotional arousal ratings (F(1, 101) = 587.45, p < 0.0001,  $\eta_p^2 = 0.853$ ), and startle reflex (F(1, 100) = 10.14, p < 0.01,  $\eta_p^2 = 0.094$ ). Unpleasant scenes were assessed as being more negative, provoking greater emotional arousal and prompting enhanced startle reflex magnitude as compared to neutral pictures (Table 2).

For affective valence and arousal ratings, the main effects of HRV (F(1, 101) = 3.26, p = 0.07,  $\eta_p^2$  0.031 and F(1, 101) = 0.42, p = 0.52,  $\eta_p^2 = 0.004$ ) and the 2-way interaction of the Picture category × HRV (F(1, 101) = 0.57, p = 0.45,  $\eta_p^2$  0.006 and F(1, 101) = 0.16, p = 0.74,  $\eta_p^2 = 0.001$ ) were not found to be statistically significant. Comparable results were found for eyeblink magnitude. Specifically, the main effect of HRV and the interaction between the Picture category × HRV were again not significant (F(1, 98) = 1.12, p = 0.29,  $\eta_p^2$  0.011 and F(1, 98) = 3.19, p = 0.07,  $\eta_p^2$  0.032, respectively). These results suggest a similar pattern of emotion reactivity for participants irrespective of their resting HRV.

# Resting HRV and affective ratings during emotion regulation

The main effect of Cue on affective ratings was significant for both valence (F(2, 148) = 102.58, p < 0.0001,  $\eta_p^2 = 0.504$ ) and arousal (F(2, 171) = 149.36, p < 0.0001,  $\eta_p^2 = 0.597$ ) (Figure 2). As shown in Table 1, post-hoc comparisons demonstrated that trials with upregulation cues were rated as being more unpleasant, provoking greater emotional arousal as compared to the nonregulation cond ition (t(102) = 8.51, p < 0.0001, d = 0.61, and t(102) = 10.33, p < 0.0001, d = 0.63, respectively). In addition, post-hoc analyses showed that participants rated the trials with downregulation cues as being less unpleasant, prompting less emotional arousal as compared to the control condition (t(102) = 8.24, p < 0.0001, d = 0.79 and t(104) = 9.17, p < 0.0001, d = 0.56, respectively).

With regard to affective ratings, ANCOVA did not show any significant main effects of HRV with regard to valence (F(2, 101) = 1.28, p = 0.26,  $\eta_p^2 = 0.013$ ) nor arousal (F(2, 101) = 0.39, p = 0.535,  $\eta_p^2 = 0.004$ ). Similarly, the ANOVA performed for each

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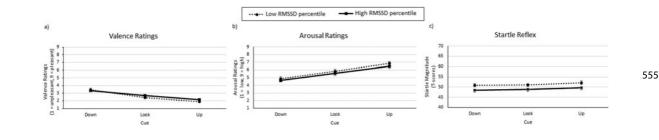


Figure 2. Results of subjective and physiological correlates of emotion reactivity and emotion regulation for each picture category and instruc-510 560 tion, depending on the participants RMSSD values. (a) Means of hedonic valence; (b) Means of emotional arousal; (c) Means of startle magnitude. High and low RMSSD values were obtained from the calculation of 3 percentiles.

affective dimension did not show any significant effects of the interaction between the Cue and HRV 515 with respect to valence (F(2, 148) = 2.11, p = 0.124) $\eta_p^2$  0.02) nor arousal (F(2, 171) = 0.68, p = 0.48,  $\eta_p^2$  = 0.07).

#### Resting HRV and startle reflex during emotion 520 regulation

The overall ANCOVA revealed a significant main effect of Cue (F(2, 196) = 8.65, p < 0.0001, = 0.081) on the startle reflex (Figure 2). Specifically, eyeblink magni-525 tude was enhanced during the upregulation of negative emotions as compared to nonregulation (t (99) = 3.49, p < 0.001, d = 0.33). However, no significant differences were found with respect to the nonregulation condition when participants were instructed to 530 downregulate their negative emotions (t (99) = 0.13, p = 0.89, d = 0.01 (see Table 3).

> The main effect of HRV was significant (F(1, 98) =7.72. p < 0.01, = 0.073). Specifically, an inverse relationship was found between HRV and startle

Table 3. Means (± SD) and confidence intervals (CI) for affective ratings and startle reflex during emotion regulation separately for each cue condition.

		Overall		
		95% CI		
	Mean (SD)	Lower	Upper	
Valence ratings				
Down	3.33 (.97)	3.14	3.52	
Look	2.60 (0.86)	2.43	2.77	
Up	2.09 (0.82)	1.94	2.25	
Arousal ratings				
Down	4.88 (1.53)	4.59	5.18	
Look	5.75 (1.56)	5.44	6.05	
Up	6.70 (1.45)	6.42	6.99	
Startle reflex				
Down	50.02 (4.12)	49.22	50.82	
Look	49.97 (3.54)	49.27	50.66	
Up	51.41 (5.04)	50.44	52.39	

reflex for upregulation (B = -2.72, 95% interval confidence [-4.8, -0.65], *t* = -2.61, *p* < 0.05), downregula-565 tion (B = -1.96, 95%) interval confidence [-3.66,-0.25], t = -2.28, p < 0.05), and nonregulation (B =-1.48, 95% interval confidence [-2.96, -0.01], t = -1.99, p < 0.05), showing that higher HRV was related to decreased startle reflex responses. The 570 interaction of Cue × HRV for startle reflex was not significant (F(2, 196) = 1.06, p = 0.35, = 0.011). These results suggest that the voluntary modulation of eyeblink responses was not influenced by HRV overall nor for each particular cue condition.

#### Discussion

The current research aimed to explore the influence of resting HRV on voluntary cognitive reappraisal during 580 the down- and upregulation of negative emotions prompted by unpleasant pictures.

In relation to emotional reactivity to different types of affective valence, our results showed that, irrespective of HRV, participants rated unpleasant pictures as being more negative and arousing as compared to neutral images. Participants also showed greater eyeblink responses when presented with negative pictures. Considering their emotional responses, the current findings do not support the notion that self-590 reported and startle reflex responses to neutral and unpleasant pictures are associated with resting HRV values. These findings are not in accordance with those of prior studies, which showed that participants with low HRV had difficulties distinguishing safe 595 (neutral stimuli) from threatening stimuli, whereas those with high HRV presented greater startle modulation (Ruiz-Padial et al., 2003; Ruiz-Padial & Thayer, 2014). In addition, our results revealed that HRV was inversely related to startle reflex magnitude, 600 suggesting that HRV is linked to defensive responses.

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This in turn could explain why individuals with lower HRV reacted with enhanced startle reflex responses.

Regarding the effects of voluntary ER, our findings were partially consistent with those of prior work. Specifically, we replicated the expected pattern of 605 affective ratings during regulation (down < look < up) for both valence and arousal (Bernat et al., 2011). With regard to startle reflex, enhanced eveblinks were found when participants were instructed to voluntarily increase their ongoing negative 610 emotions, in line with the findings of past studies (Bernat et al., 2011; Dillon & LaBar, 2005). However, we did not replicate the diminished blink responses when participants were instructed to decrease the emotions prompted by unpleasant and arousing pic-615 tures. Although these results have been discussed in greater detail in our previous work (Fuentes-Sánchez et al., 2019), we emphasise that our results are in line with those of previous studies that also found difficulties in demonstrating a significant reduction 620 in startle reflex when participants decreased their negative emotions (Bernat et al., 2011; Dillon & LaBar, 2005).

With regard to the moderating effect of HRV in the 625 relationship between reappraisal and startle reflex modulation, some distinctions between RMSSD and HR-HRV results were found. The differences between the measures may be explained by the different statistical properties of RMSSD and HF-HRV and their differential sensitivity to vagal differences. Specifically, 630 our results using RMSSD values do not support the idea that the pattern found for self-reported and startle reflex responses for each ER instruction is affected by HRV values. These results may have some clinical implications, and fit well with prior 635 studies that failed to find an association between vagal HRV and cognitive performance (Duschek et al., 2009; Jennings et al., 2015). In particular, since ER strategies such as cognitive reappraisal are useful for managing negative emotions (Zaehringer et al., 640 2020), the findings suggest that individuals could benefit from these strategies regardless of their HRV characteristics. Regarding the analyses performed with HF data (Supplemental 1), our findings suggest a different pattern in the effectiveness of emotion 645 regulation for subjective valence ratings. As such, individuals with lower HRV could benefit more greatly from emotion regulation instructions as compared to those with high heart rate variability. The current findings may suggest that the plausible differences 650 between "good" and "bad" emotion regulators are not derived from the implementation of strategies to regulate emotions when suitable methods are provided. These differences may in fact be caused by difficulties at earlier stages, such as in the identification or selection of specific strategies to manage negative circumstances (Sheppes et al., 2015). Thus, people with low HRV could improve their competencies in voluntary ER when they are provided with appropriate strategies to cope with particular events. However, it is important to note that the limited power of the study prevents us from drawing robust conclusions in this regard.

One limitation of the present study concerns the characteristics of the sample. Here, we have examined the effects of cognitive reappraisal in healthy participants, which may not necessarily be generalisable to clinical populations. Furthermore, the ER task may have been affected by the instructions given to participants. In particular, if HRV is considered a measure of psychological flexibility, the fact that participants were asked to use cognitive reappraisal with specific instructions – as opposed to using their own natural language - could have impacted negatively on the observation of their real ability to regulate their emotions (Gyurak et al., 2012). Accordingly, 675 future studies should investigate the associations between HRV and ER using more flexible experimental designs focused on the selection of suitable strategies in real-life contexts and in different ER stages, for example through ecological momentary assess-680 ment using technology (Colombo et al., 2020). Moreover, clinical samples should also be explored in order to provide results that could be applicable to psychological interventions. Nevertheless, our findings support the idea that ER might be relatively easy to 685 learn and implement in short-term experimental settings, which is encouraging for applied clinical research.

#### Notes

- 1. IAPS numbers used in this experiment: Unpleasant (6313; 3500; 2683; 6571; 6212; 6540; 6530; 9163; 6834; 9252: 9426; 9423; 9635; 9452; 6560; 6315; 2691; 6312; 6211; 6520; 3530; 6550; 6350; 6360; 9414; 9425; 9427; 6821; 9428; 9413; 2710; 2141; 2900; 3350; 3061; 3010; 3181; 9250; 3550; 9050; 9400; 3301; 9910; 9920; 9530; 3191; 3150; 9254; 2053; 3300; 3101; 3060; 3230; 6022; 9421; 9420; 9435; 9908; 9412; 9921); Neutral (7004; 7233; 7009; 7010; 7025; 7080; 7035; 7235; 7175; 7006).
- 2. The Kolmogorov-Smirnov test performed a priori indicated that the raw RMSSD values before the log transformation did not follow a normal distribution (D [112]

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= 0.12, p < 0.0001). After the log transformation, RMSSD values followed a normal distribution (D [112] = 0.04, p= 0.2).

#### 705 **Disclosure statement**

**Q3** No potential conflict of interest was reported by the author(s).

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#### Author statemen

All authors were strongly involved in the study and 720 reviewed and discussed the manuscript. IJ and NFS prepared the first draft of the manuscript, which was then reviewed by MCP, MAE, GRP, and CSR. MCP, IJ, NFS, and MAE managed sample recruitment and physiological data reduction, while CSR and GRP 725 actively participated in statistical analysis and manuscript revision.

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