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## **The continuum of consciousness in cardiovascular stress research : an experimental expedition**

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# Chapter 3

## Cardiovascular activity in response to subliminal presentation of threatening and neutral words

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## Abstract

Stress-related cognitive processes may occur outside of awareness, here referred to as unconscious stress, and affect one's physiological state. Evidence supporting this idea would provide necessary clarification of the relationship between psychological stress and cardiovascular (CV) health problems. We tested the hypothesis that increases in mean arterial pressure (MAP) and total peripheral resistance (TPR) and decreases in heart rate variability (HRV) would be larger when threatening stimuli are presented outside of awareness, or subliminally, compared with neutral stimuli. Additionally, it was expected that trait worry and resting HRV, as common risk factors for CV disease, would moderate the effect. We presented a subliminal semantic priming paradigm to college students that were randomly assigned to the threat ( $n = 56$ ) or neutral condition ( $n = 60$ ) and assessed changes from baseline of MAP, TPR, and HRV. Level of trait worry was assessed with the Penn State Worry Questionnaire. The findings indicate that CV activity changed according to the hypothesized pattern: A higher MAP and TPR and a lower HRV in the threat condition compared with the neutral condition were found with practically meaningful effect sizes. However, these findings were only statistically significant for TPR. Furthermore, changes in CV activity were not moderated by trait worry or resting HRV. This is the first study to explicitly address the role of subliminally presented threat words on health-relevant outcome measures and suggests that unconscious stress can influence peripheral vascular resistance.

Perseverative cognition (e.g., worry) is associated with continuous adverse physiological activation (18,20,21) and the development of cardiovascular (CV) disease (e.g., 201). However, the underlying mechanisms remain elusive. Perseverative cognition has been referred to as a form of ongoing stress-related cognition (17,18). It has been suggested that stress-related cognition might also occur outside of awareness, here referred to as unconscious stress, and as a consequence affects physiological states (26,27). This is supported by the inability of self-report measures of affective states to fully explain physiological responses to stressful experiences (23-25). In other words, individuals may not be aware of a higher level of psychological stress even though they could be facing adverse physiological activation.

Self-report is just one type of measurement, where subjects are directly asked about a construct, while other types of measurement, such as behavioral outcomes or indirect measurement of the construct of interest, might be of equal importance (47,66). The findings of several experimental studies that used indirect measures, in addition to self-reported affect, indicate that affect measured indirectly can explain physiological responses to stress (76,82,202). For example, in the study performed by Van der Ploeg et al. (2016, 202), the Implicit Positive and Negative Affect Test was related to changes in CV activity in response to a stressor independently from the self-report measure of negative affect. Comparable relationships with CV activity were found for a dot probe task (82) and the Implicit Association Test with an anxiety-specific version (76). Thus, it appears that the relationship between psychological stress and health might at least partly be explained by unconscious processes.

Yet, the relationship between unconscious stress and physiology has been primarily supported by correlational studies (203). Only a handful of studies looked at the effect of subliminal priming using stress-related primes on CV outcome measures. For example Garfinkel et al. (2016, 61) and Hull, Slone, Meteyer, and Matthews (2002, 62) used subliminal semantic priming and found that the prime word 'angry' compared with the word 'relax' increased systolic and diastolic blood pressure (BP) and heart rate (HR). Furthermore, Levy, Hausdorff, Hencke, and Wei (2000, 198) also used subliminal semantic priming and found increases in CV activity after priming older individuals with words containing negative stereotypes of aging, as opposed to positive stereotypes. These findings suggest that subliminally primed stress-related words may affect CV activity, but the findings are still small in number. Moreover, the stimuli used have not been validated as threatening words in earlier studies (e.g., 204). Furthermore, in these studies the results of the negatively valenced primes were compared with those of positive primes that constitute suboptimal control stimuli compared with neutral primes. Although the findings of the described studies suggest that words presented subliminally may alter CV activity, the studies used very specific concepts, such as the word 'angry', that cannot be readily translated into the more general concept of *stress* in a healthy population. Consequently, the generalizability of the

findings is limited, and they do not sufficiently clarify the relationship between stress and health. Therefore, in the current study we addressed whether stress-related cognitions induced outside of awareness through semantic subliminal priming of threat-related words can affect CV activity.

Furthermore, we aimed to test whether these possible effects of subliminal threatening information on CV activity would be particularly strong in people at risk of developing CV health problems. More specifically, we were interested in these effects in people high in trait worry (201) and low in resting heart rate variability (HRV; 205,206). With respect to the first suggested moderator, trait worry, some individuals have the disposition to worry excessively. High trait worry is typically observed in patients suffering from generalized anxiety disorder (GAD; 207-209), which has been related to adverse CV activity in experimental settings (210,211), an increased risk for the development of CV disease in the general population (212), and acute cardiac events in patients with stable coronary heart disease (213,214). Furthermore, high trait worriers compared with low trait worriers have been found to display lower levels of resting HRV (215) and show a delayed CV recovery after a stressor (90). Additionally, in high trait worry females, a higher HR during stressful tasks was found compared with low trait worry female controls (216). Importantly, worriers tend to display a bias toward threatening information and interpret ambiguous information as threatening, both without awareness or intent (217-221). When a source of threat is presented to worriers, their preexisting cognitive representations of threat are activated and influence task-related behavior (219). Overall, this suggests that trait worriers are more sensitive to threatening information that, by prolonging the physiological stress response, puts them at risk for CV disease (21). Thus, trait worry may play a moderating role between unconscious stress and CV physiology, and its contribution is examined in the current study.

With respect to the second moderator, low HRV is a CV outcome crucial to disease risk (205,206,222). Moreover, a series of studies has shown that low HRV also *predicts* physiological and cognitive responses to threat-related manipulations by influencing emotional attention (223-227). In their review, Park and Thayer (2014, 225) suggest that, whereas high resting HRV indicates flexible and adaptive cognitive functioning, low resting HRV indicates impaired cognitive processing. This implies that resting HRV may be used as a biological marker of maladaptive emotional and physiological responding. Therefore, in the current study, the moderating role of resting HRV was considered in the effect of threat on CV and behavioral outcome measures.

In sum, the current study was conducted to test whether unconscious stress affects health-related physiology by using a subliminal semantic priming paradigm with threatening and neutral words. Additionally, we tested whether this relation was moderated by participants' level of trait worry and resting HRV. We expected stronger CV responses, that is, larger increases in mean arterial pressure (MAP) and

total peripheral resistance (TPR) and larger decreases in HRV, in participants in the threat condition compared with the neutral condition and expected that this effect was particularly observed in participants with high levels of trait worry or low resting HRV.

## Method

### Participants

Students enrolled in an introductory course in psychology signed up for the experiment and received course credit after participation. We assessed whether they refrained from drinking caffeine in the four hr and exercising in the two hr prior to the experiment as requested before the experiment started, and confirmed this subsequently using the biobehavioral questionnaire. Of the 136 participants that were tested, two did not adhere to these requests and showed deviating CV activity. Twelve individuals reported current CV or psychological health problems, such as Attention Hyperactivity Disorder and GAD, and/or those using medication for such problems on the biobehavioral questionnaire and were excluded. In five cases the experiment failed due to technical problems, and one participant withdrew consent during testing. This resulted in a final sample of 116 participants (age:  $M = 19.1$ ,  $SD = 1.81$ ; 55.2% female). They were allocated randomly, using an online generator (<http://www.graphpad.com/quickcalcs/randomize2/>), to the threat ( $n = 56$ ) or neutral condition ( $n = 60$ ). The experiment was approved by the Institutional Review Board (2010B0035) of The Ohio State University, Columbus, Ohio.

### Instruments

#### **Cardiovascular activity**

BP (mmHg) was recorded with the Finometer Model 2 (Finapres Medical SystemsBV, Amsterdam, The Netherlands) and checked for artifacts in Beatscope 1.1.0.6, which was also used to extract MAP (mmHg). Furthermore, the electrocardiogram (ECG) and impedance cardiograph (ICG) were continuously recorded with MindWare 2000D Impedance Cardiograph package in BioLab 3.0.13 at a sample rate of 1000 Hz. The data were checked for artifacts and processed in MindWare 3.0.25 for HRV (116) and ICG (189). Artifacts were detected automatically by this software package, and accuracy of this detection was confirmed by visual inspection. From this data, mean values of HR (bpm), cardiac output (CO; l/min), basal impedance ( $Z_0$ ; ohms), the impedance peak ( $dZ/dt$ ; ohms/s), and interbeat interval (IBI; ms) were obtained. IBIs were used to determine the mean root mean squared successive differences (RMSSD; ms) in Kubios HRV 2.2, and the data were prepared for data analysis using a macro for Office Excel (228). TPR (mmHg.min/L) was calculated from MAP and CO (229,230). The computer on which the physiological data were collected was not connected to the computer on which the priming task was performed, and markers were placed

manually. As a consequence, it was considered inaccurate to extract the data for each trial. Baseline values of MAP, RMSSD (i.e., resting HRV), and TPR consisted of the mean activity during the last two min of the baseline. Mean MAP, RMSSD, and TPR during the entire priming task were additionally calculated.

### **Questionnaires**

Trait worry was measured with the Penn State Worry Questionnaire (PSWQ), which consists of 16 self-report items that are answered on a five-point scale. Strong internal consistency and overall validity have been established (208,231). In the current sample, the PSWQ was found to be reliable with Cronbach's  $\alpha = .94$ .

The effect of the primes on affect was measured indirectly with an implicit measure of affect, the Implicit Negative and Positive Affect Scale (IPANAT; 84), which has been found to be associated with stress-related CV activity (202). The IPANAT is designed as an affect misattribution task and asks participants to evaluate the resemblance of a series of nonsense words to 12 emotional adjectives (joyful, annoyed, afraid, sad, cheerful, irritated, frightened, happy, gloomy, angry, scared, unhappy) on a six-point scale where 1 indicates "does not fit at all" and 6 "fits very well". The scores are averaged into two subscales: Implicit negative affect (INA) and implicit positive affect (IPA). In the original format, six artificial words (safme, vikes, tunba, taleb, belni, sukov) are presented. In this study 'safme' was left out after previous studies showed that it often reminded participants of 'save me' (202), which probably has an affective association and was likely to confound the results. Quirin et al. (2009, 84) established adequate internal consistency, strong convergent and discriminant validity, and strong sensitivity to momentary affective manipulation. In the current study, the INA and IPA subscales were found to be reliable with Cronbach's  $\alpha = .87$  and Cronbach's  $\alpha = .92$ , respectively. This is comparable to previous studies (e.g., 84,202).

### **Subliminal priming task**

To induce a threatening or neutral state we used a subliminal semantic priming task during a Lexical Decision-making Task (LDT), which is a common way to establish a subliminal priming effect (e.g., 62,67,232,233). Depending on condition, participants were presented with 24 threatening or 24 neutral prime words as primes during 48 trials. All primes were presented twice in random order. The threatening words have been shown to elicit different behavioral and physiological responses in anxious and nonanxious individuals (204,211). The neutral words were selected from the Affective Norms for English Words (ANEW; 234) pool of words by level of arousal and matched with the threatening words by word length. The list of prime words can be found in Table 1. The target words for the LDT, "words" or "nonwords", were also selected from the ANEW set of words, but were different from the prime words. The words were neutral and low in arousal (e.g., glass, listless). The nonwords were scrambled (e.g.,



rfinioer, vcpeae, using [textmechanic.com/Word-Scrambler.html](http://textmechanic.com/Word-Scrambler.html)) versions of different neutral words with a maximum arousal level of four (234). During the task, 24 words and 24 nonwords were randomly selected from two lists of 96 possible targets for both types of targets. The task was preceded by eight practice trials with different scrambled neutral words as primes. Target words were only presented once during practicing and the task itself.

Each trial started with a fixation cross that was shown for 500 ms in the middle of the screen, followed by a prime word that was shown for 17 ms (similar to 62,64). The prime was asymmetrically “sandwich masked” with a forward (300 ms) and backward mask (33 ms), both consisting of a string of XXXs (67,233,235). This procedure leads to the visual appearance of flashes, which was included in the instructions as indicating that the target word was about to appear. The backward mask was immediately followed by the target word. Participants were instructed to indicate whether they saw a word or nonword as fast as they could, by pressing *F* or *K*, respectively, on a keyboard

**TABLE 1** List of prime words used in the study

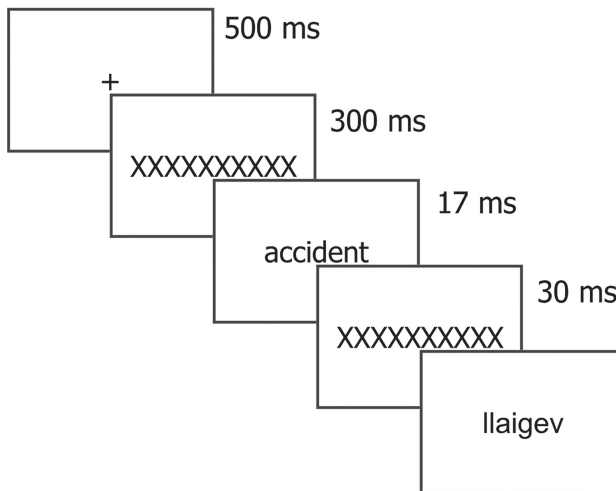
Threat prime	Neutral prime
disease	hairpin
injury	bathroom
coronary	bland
mutilated	board
fatal	corner
ambulance	corridor
coffin	curtains
hazard	windmill
cancer	errand
deathbed	hairdryer
emergency	haphazard
paralyzed	icebox
indecisive	indifferent
pathetic	mantel
foolish	nonchalant
lonely	pamphlet
inferior	plain
criticized	reserved
inept	kerchief
hated	solemn
inadequate	square
stupid	stomach
failure	subdued
embarrassed	thermometer

**Note.** The prime words were presented twice

indicated with yellow stickers. No feedback was provided on accuracy or speed to prevent confounding effects of the feedback. A new trial started immediately after the response or 1490 ms after target onset. See Figure 1 for a schematic display of a trial. The task was executed on a CRT screen with a resolution of 800 x 600 pixels and the refresh rate was set at 60 Hz. The baseline, practice and task were programmed in DirectRT v2006.20.28.

Reaction times (RT) to the targets faster than 100 ms and slower than 1250 ms, incorrect responses (4.11%), and responses three times the individual SD were excluded from further analysis (236). Data of participants with over 25% of invalid responses were not included in the final analysis (3.45% of the final sample). Mean RT were calculated across trials and for word and nonword trials separately.

To check for prime detection, we provided a forced choice prime recognition task during which all prime words, threat and nonthreat, were presented (e.g., 121). Participants had to indicate, with *yes* or *no*, whether they remembered seeing any of the words during the experiment. The sensitivity measure  $d'$  and its 95% confidence intervals were calculated from the responses based on signal detection theory. Participants were assumed to have been able to discriminate between the stimulus types when their  $d'$  measure 95% confidence intervals would not include zero (237). Furthermore, valence and arousal levels for all prime words were assessed on a Visual Analog Scale, with zero = *negative or not arousing at all* and 100 = *positive or very arousing*.



**FIGURE 1** Depiction of the subliminal semantic priming procedure. Each trial started with a fixation cross (500 ms), followed by a forward mask (300 ms), a threatening or neutral prime (17 ms), a backward mask (30 ms), and a word/nonword as target that was presented until the participant responded or 1,490 ms had passed

## Procedure

After an explanation of the procedure, participants provided informed consent and were attached to the physiological recording apparatus. They were seated in a comfortable chair facing the middle of the CRT screen. The experiment started with a baseline measurement of five min during which participants were instructed to try to relax and do nothing. This was followed by instructions for the priming task, the practice trials, and the task. The questionnaires and forced choice prime recognition task (using MediaLab v2012.4.133) and the valence and arousal ratings (using E-prime 2.0) were displayed afterward on a different screen that was turned on after the priming task. Participants were carefully debriefed about the goal of the study and presentation of prime words. The experiment took about one hr and was conducted in a laboratory suited for psychophysiological measurements.

## Statistical analyses

In several cases technological difficulties (i.e., equipment failure, unusable BP, ECG, or ICG data, and overwritten data) prevented adequate measurement of one or more physiological outcome measures. These data were considered to be missing at random. CV reactivity to the primes was expressed in change scores (i.e., mean activity during the entire priming task minus the last two min of the baseline; 188,238). The change scores, calculated only for participants with data at rest and during the task, were checked for outliers ( $> 3$  SDs from the group mean). Differences in baseline biobehavioral characteristics (including baseline MAP, TPR, and RMSSD) between conditions were analyzed with  $t$  tests for continuous variables and chi-square tests for categorical variables. Differences between conditions (threat versus neutral primes) in the results of the CV outcomes, forced choice prime recognition task, valence and arousal ratings of the primes, and RT to the targets were analyzed with one-sided  $t$  tests as we expected stronger changes and differences in the threat condition. Effect sizes are expressed in  $r$  (239). As main analyses, we performed three separate moderation analyses with MAP, TPR, and HRV as dependent variables for both moderators, trait worry and resting HRV. Condition was a predictor in all analyses. In all models predictor variables were centered, and bootstrapping for indirect effects was applied with 1,000 samples. All analyses were performed with SPSS 23.0 using the PROCESS macro for SPSS for the moderation analyses (240).

## Results

Two participants displayed BP values that were considered to be extreme (systolic BP  $> 175$  and diastolic BP  $> 110$ ), and the related BP variables were not included in the analysis to be conservative. The  $d'$  measure 95% confidence intervals included zero for all participants, and it was consequently assumed that none of them had

been able to discriminate between the two prime types (237). This implies that the primes were successfully presented subliminally. The final number of cases for each outcome measure and other baseline characteristics are presented in Table 2. There were no differences between conditions, but a marginally significant lower number of participants smoked in the threat condition compared with the neutral condition. Analyses were performed with and without smoking status as covariate in the analyses regarding the CV variables. This did not alter the findings, and results without smoking status are reported. Trait worry was normally distributed with  $M = 50.1$  ( $SD = 14.2$ ) and was higher in females ( $M = 54.5$ ,  $SD = 14.6$ ) than males ( $M = 44.8$ ,  $SD = 11.7$ ,  $t(113) = 3.89$ ,  $p < .001$ ), which is comparable to previous (student) samples (208). Resting HRV was normally distributed with  $M = 47.1$  ( $SD = 24.4$ , range 4.91; 125.0), which is

**TABLE 2** Baseline characteristics stratified by condition

Measure	Threat			Neutral			t/ $\chi^2$
	M	SD	n	M	SD	n	
<i>Demographics</i>							
Age, years	18.9	1.41	56	19.2	2.11	60	-1.12
Female sex <sup>a</sup>	29	(52)	56	35	(58)	60	0.50
BMI	24.9	5.00	56	24.3	4.93	60	0.69
White Caucasian <sup>a</sup>	47	(84)	55	46	(75)	60	1.96
<i>Biobehavioral variables</i>							
Smoking <sup>a</sup>	0	(0)	55	3	(5)	60	2.82 <sup>+</sup>
Caffeine use (last 24 hr)	2.56	6.45	55	2.14	6.36	59	0.36
Alcohol use (glass/last 24 hr)	0.02	.135	55	0.05	.29	60	-0.75
Exercise (last 24 hr) <sup>a</sup>	22	(39)	55	32	(53)	60	2.05
<i>Cardiovascular measures</i>							
SBP	117.0	15.4	54	121.7	17.9	56	-1.49
DBP	63.0	13.3	54	66.4	12.2	56	-1.40
MAP	78.2	11.3	54	81.9	12.3	56	-1.67
HR	73.1	11.1	55	73.8	11.5	56	-0.36
RMSSD	48.1	25.1	55	46.1	23.8	55	0.43
TPR	10.8	4.63	49	11.2	4.12	47	-0.38
<i>Personality</i>							
Trait worry	50.1	14.3	55	50.0	14.1	60	0.04

**Note.** The cell sizes are displayed since the amount of usable recordings varied across outcome measures. All tests were performed two-sided. There were no significant differences between the conditions. BMI = Body mass index, DBP = Diastolic blood pressure, HR = Heart rate, MAP = Mean arterial pressure, RMSSD = Root mean square of successive differences, SBP = Systolic blood pressure, TPR = Total peripheral resistance.

<sup>a</sup> Indicated with number of positive responses (percentage), Pearson  $\chi^2$  was used as test statistic.

<sup>+</sup>  $p < .10$

comparable to previous studies (241). No differences between males ( $M = 46.3$ ,  $SD = 25.3$ ) and females ( $M = 47.8$ ,  $SD = 24.6$ ,  $t(108) = 0.313$ ,  $p = .755$ ) were found.

### **Valence and arousal words**

The threatening words were rated as more negative ( $M = 21.1$ ,  $SD = 7.19$ ) compared with the neutral words ( $M = 3.79$ ,  $SD = 1.70$ ;  $t(65) = 9.82$ ,  $p < .001$ ,  $r = .77$ ). The threatening words also elicited a higher feeling of arousal ( $M = 21.1$ ,  $SD = 7.19$ ) compared with the neutral words ( $M = 49.6$ ,  $SD = 3.68$ ,  $t(68) = -30.8$ ,  $p < .001$ ,  $r = .97$ ), using a square root transformation to address the positively skewed distribution of the data on arousal to neutral words. No differences between conditions were apparent in mean ratings of valence and arousal of the threatening words,  $t(67) = -0.534$ ,  $p = .595$ ,  $r = .065$ ;  $t(64) = -1.36$ ,  $p = .177$ ,  $r = .17$ , respectively, nor in those of the neutral words,  $t(67) = -0.760$ ,  $p = .450$ ,  $r = .092$ ;  $t(67) = 0.912$ ,  $p = .365$ ,  $r = .11$ , respectively.

### **Cardiovascular reactivity**

The mean MAP was higher during the performance of the priming task ( $M = 82.6$ ,  $SD = 13.1$ ) compared with baseline ( $M = 80.1$ ,  $SD = 11.9$ ;  $t(109) = -5.11$ ,  $p < .001$ ,  $r = .44$ ). Mean RMSSD did not change from baseline ( $M = 47.1$ ,  $SD = 24.4$ ) to the priming task ( $M = 46.9$ ,  $SD = 23.5$ ;  $t(109) = 0.15$ ,  $p = .885$ ,  $r = .01$ ). Furthermore, mean TPR was higher during the priming task ( $M = 11.7$ ,  $SD = 4.78$ ) compared with baseline ( $M = 11.0$ ,  $SD = 4.38$ ;  $t(94) = -2.45$ ,  $p = .016$ ,  $r = .24$ ).

The mean MAP reactivity in the threat condition ( $M = 3.10$ ,  $SD = 3.83$ ) did not differ from the neutral condition ( $M = 2.42$ ,  $SD = 4.56$ ;  $t(107) = 0.84$ ,  $p = .405$ ,  $r = .08$ ). Similarly, the mean RMSSD reactivity in the threat condition ( $M = -1.54$ ,  $SD = 13.2$ ) did not differ from the neutral condition ( $M = 1.15$ ,  $SD = 14.9$ ;  $t(108) = -1.00$ ,  $p = .318$ ,  $r = .10$ ). Finally, the mean TPR reactivity in the threat condition ( $M = 0.942$ ,  $SD = 1.55$ ) significantly differed from the neutral condition ( $M = 0.257$ ,  $SD = 2.17$ ;  $t(92) = 1.76$ ,  $p = .041$ ,  $r = .18$ ). Thus, all CV activity changed in the expected direction with practically meaningful effect sizes, but statistically significantly so only for TPR.

### **Task performance**

The mean RT during the priming task in the threat condition ( $M = 678.3$ ,  $SD = 87.8$ ) did not differ from the neutral condition ( $M = 652.6$ ,  $SD = 88.8$ ;  $t(105) = 1.51$ ,  $p = .134$ ,  $r = .15$ ). Similarly, no differences between conditions were found for the words (threat:  $M = 674.7$ ,  $SD = 100.7$ ; neutral:  $M = 652.1$ ,  $SD = 104.7$ ;  $t(105) = 1.14$ ,  $p = .258$ ,  $r = .11$ ) and the nonwords (threat:  $M = 724.2$ ,  $SD = 130.6$ ; neutral:  $M = 695.4$ ,  $SD = 125.4$ ;  $t(105) = 1.16$ ,  $p = .247$ ,  $r = .11$ ).

### Moderating effects of trait worry and resting HRV

We expected that the effect of the primes on CV reactivity would be positively related to trait worry and negatively related to resting HRV. Results of the moderation analyses are displayed in Table 3 and 4. The simple moderation analysis with trait worry as moderator showed a small but nonsignificant interaction effect with condition on mean MAP reactivity ( $b = 0.020$ , 95% bootstrapped CI [-0.110, 0.151],  $t(104) = 0.31$ ,  $p = .757$ ,  $r = .30$ ). There was no effect for mean TPR reactivity ( $b = 0.002$ , 95% bootstrapped CI [-0.049, 0.052],  $t(89) = 0.059$ ,  $p = .953$ ,  $r = .006$ ) and mean RMSSD reactivity ( $b = -0.055$ , 95% bootstrapped CI [-0.473, 0.364],  $t(105) = -0.26$ ,  $p = .796$ ,  $r = .03$ ). Furthermore, none of the models explained a significant portion of the variance in CV reactivity. Thus, the effect of the primes on TPR and RMSSD reactivity was not dependent on trait worry, but a small effect on MAP was apparent.

The simple moderation analysis with resting HRV as moderator showed a small but significant negative association between resting HRV and mean RMSSD reactivity ( $b = -0.196$ , 95% bootstrapped CI [-0.331, -0.060],  $t(106) = -2.88$ ,  $p = .005$ ,  $r = .27$ ) and a small marginally significant positive association between resting HRV and mean MAP reactivity ( $b = 0.026$ , 95% bootstrapped CI [-0.005, 0.056],  $t(99) = 1.66$ ,  $p = .099$ ,  $r = .16$ ). The interaction effect with condition was absent for mean MAP reactivity ( $b = -0.004$ , 95% bootstrapped CI [-0.065, 0.058],  $t(99) = -0.11$ ,  $p = .910$ ,  $r = .01$ ), TPR reactivity ( $b = -0.01$ , 95% bootstrapped CI [-0.045, 0.017],  $t(89) = -0.90$ ,  $p = .373$ ,  $r = .09$ ), and RMSSD reactivity ( $b = -0.083$ , 95% bootstrapped CI [-0.353, 0.187],  $t(106) = -0.61$ ,  $p = .545$ ,  $r = .008$ ).

Additionally, the models of MAP and TPR reactivity were not significant, but the model of mean RMSSD reactivity was significant,  $F(3, 106) = 3.44$ ,  $p = 0.019$ , and explained 13.2% of the variance. Thus, the effect of the primes on CV reactivity was not moderated by resting HRV, but higher resting HRV levels were associated with increases in MAP and decreases in RMSSD in both conditions.

### Exploratory analyses

An exploratory moderation analysis of the effect of the primes on CV reactivity  $\times$  Gender was examined. The simple moderation analysis with gender as moderator (not centered) did not show a moderation of the effect of condition on mean MAP reactivity,  $b = -0.53$ , 95% bootstrapped CI [-3.96, 2.89],  $t(105) = -0.31$ ,  $p = .759$ ,  $r = .03$ , and a small but nonsignificant effect on mean RMSSD reactivity,  $b = 6.39$ , 95% bootstrapped CI [-5.14, 17.9],  $t(108) = -1.10$ ,  $p = .274$ ,  $r = .11$ . Furthermore, the mean TPR reactivity showed a small marginally significant interaction between condition and gender,  $b = 1.59$ , 95% bootstrapped CI [-0.088, 3.26],  $t(90) = 1.88$ ,  $p = .063$ ,  $r = .18$ . In men, results showed a small marginally significant effect,  $\beta = -5.96$ ,  $SE = 3.13$ ,  $t(90) = -1.90$ ,  $p = .060$ ,  $r = .20$ , of threat words on TPR reactivity, while no such effect was found for women,  $\beta = 0.55$ ,  $SE = 4.90$ ,  $t(90) = 0.087$ ,  $p = .931$ ,  $r = .009$ . This would

indicate that in females TPR was similar in response to threatening and neutral words, whereas males displayed a lower TPR response to neutral words. This was confirmed through further exploration using simple effects analysis.<sup>1</sup> However, none of the models explained a significant portion of the variance in CV reactivity. Overall, the effect of the primes on CV reactivity was largely independent of gender.

**TABLE 3** Moderation analysis of trait worry on the effect of condition on each outcome measure

	MAP (mmHg)			TPR (mmHg.min/L)			RMSSD (ms)		
	B	SE	t	B	SE	t	B	SE	t
Constant	2.78	0.42	6.61***	0.62	0.20	3.11**	-0.08	1.37	-0.06
Condition	0.74	0.84	0.88	0.76	0.40	1.89 <sup>+</sup>	-2.60	2.74	-0.95
Trait worry	0.01	0.03	0.29	0.02	0.01	1.18	0.14	0.11	1.33
Condition × Trait worry	0.02	0.07	0.31	-0.002	0.03	0.06	-0.05	0.21	-0.26
F	0.33			1.60			1.50		
df	3, 103			3, 89			3, 105		
R <sup>2</sup>	.01			.05			.03		

**Note.** All predictor variables were centered and unstandardized regression coefficients are reported. Outcomes are expressed in change scores. MAP = Mean arterial pressure, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

<sup>+</sup> $p > .10$ , \*\* $p > .01$ , \*\*\* $p > .001$

**TABLE 4** Moderation analysis of resting HRV on the effect of condition on each outcome measure

	MAP (mmHg)			TPR (mmHg.min/L)			RMSSD (ms)		
	B	SE	t	B	SE	t	B	SE	t
Constant	2.44	0.39	6.28***	0.59	0.20	2.96**	-0.15	1.31	-0.12
Condition	1.39	0.78	1.78 <sup>+</sup>	0.73	0.40	1.83 <sup>+</sup>	-2.30	2.62	-0.88
Resting HRV	0.03	0.02	1.66 <sup>+</sup>	-0.003	0.008	-0.38	-0.20	0.07	-2.88**
Condition × Resting HRV	0.004	0.03	-0.11	-0.01	0.02	-0.90	-0.08	0.14	-0.61
F	1.66			2.05			3.44 <sup>+</sup>		
df	3, 99			3, 89			3, 106		
R <sup>2</sup>	.06			.05			.13		

**Note.** All predictor variables were centered and unstandardized regression coefficients are reported. Outcomes are expressed in change scores. MAP = Mean arterial pressure, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

<sup>+</sup> $p > .10$ , \* $p > .05$ , \*\* $p > .01$ , \*\*\* $p > .001$

<sup>1</sup> Simple contrasts indicated that men showed an increase in TPR reactivity in the threat condition compared with the neutral condition,  $t(90) = 3.17$ ,  $p = .002$ ,  $r = .32$ , whereas women showed an increase in TPR reactivity in both conditions on TPR reactivity,  $t(90) = 0.713$ ,  $p = .478$ ,  $r = .074$ . In the threat condition, both men and women showed an increase in TPR reactivity,  $t(90) = 0.330$ ,  $p = .742$ ,  $r = .035$ , whereas in the neutral condition men showed a decrease in TPR reactivity compared with women,  $t(90) = 3.48$ ,  $p < .001$ ,  $r = .34$ .

A second exploratory analysis was performed to examine the role of affect at an implicit level in the effect of the primes on CV reactivity. There was no apparent effect of condition on CV reactivity. However, recent insights suggest that limiting mediation analyses to situations where the predictor ( $X$ ) and outcome ( $Y$ ) are significantly related might be unnecessarily restrictive (e.g., 242). Moreover, inclusion of this analysis using implicit measures of affect is warranted as it is crucial for the development of the theory regarding unconscious stress and in line with previous work (202). A parallel mediation analysis was executed using ordinary least squares path analysis (240) to examine the mediation effect of the IPANAT subscales. IPA, but not INA, showed a small marginally significant relation with mean MAP reactivity,  $b = 1.09$ ,  $t(104) = 1.71$ ,  $p = .075$ ,  $r = .17$ . No relationship with mean TPR reactivity and mean RMSSD reactivity were apparent. This would indicate that IPA might be related to BP activity.

## Discussion

Unconscious stress, that part of the psychological stress response occurring outside of awareness, may provide an explanation of the relationship between psychological stress and physiological responses that ultimately lead to (CV) health problems. In the current study, the effect of unconscious stress on physiology was tested using a subliminal semantic priming paradigm with two conditions, during which either threatening or neutral stimuli were presented while measuring health-relevant CV outcome measures. Exposure to threatening stimuli, compared with exposure to neutral stimuli, was expected to elicit larger increases of MAP and TPR and a larger decrease of HRV during the task. Furthermore, the effect of this manipulation regarding characteristics that have been related to the development of CV health problems, namely, levels of trait worry and resting HRV, was examined. Subliminally presented threatening words compared with neutral words elicited a higher TPR response. No statistically significant differences between stimulus types were observed for changes in MAP and HRV, but the changes were in the expected directions and nonzero in terms of effect sizes. Furthermore, changes in CV activity were not significantly moderated by trait worry or resting HRV.

This is the first study to explicitly address the health-relevant physiological effects of validated threatening stimuli presented subliminally. The findings extend previous findings by Garfinkel et al. (2016, 61), Hull et al. (2002, 62), and Levy et al., (2000, 189) who found that subliminally presented stimuli affected CV activity. In general, these studies support the hypothesis that unconscious stress may affect CV activity (26,27), although in our study we found this to be the case only for peripheral vascular changes.

It was expected that unconscious stress would influence all CV measures, but the effects were the largest for TPR. Nevertheless, the observed differences in TPR reactivity between threatening and neutral words are in line with the biopsychosocial



model of challenge and threat (99,100), even though in this study the stimuli were presented subliminally. TPR reflects an alpha-adrenergic response that is associated with threat (99). Higher TPR has been associated with the development of hypertension leading to end organ damage (98) and increased incidence of CV events and all-cause mortality (97). Moreover, the effects on TPR corroborate previous findings that (ongoing) stress-related cognitions are associated with a more vascular activation pattern, that is, changes in indices of vascular function such as total peripheral resistance rather than indices of cardiac changes such as cardiac output (202,243). Although in the current experiment both conditions showed an increased TPR response to the primes, indicating task engagement (100), it was relatively higher in the threat condition. This indicates that even the subtle presentation of threat through subliminal priming elicited an increase in peripheral vascular activity. In other words, although the reader should keep in mind that the effect was small, unconscious stress seems to affect a clinically relevant physiological parameter.

The subliminal threatening primes induced small changes ( $r_s < .15$ ) in other CV parameters in the expected directions, which are practically meaningful. Similar studies have shown BP and HR changes to subliminal primes (61,62,198), but found larger effects compared with this study ( $r_s > .30$ ). Moreover, the study by Garfinkel et al. (2016, 61) reported larger effects on the behavioral outcomes (i.e., RTs;  $r_s > .50$ ) than in the current study ( $r = .15$ ). This indicates that the differences between conditions in the current study were smaller compared with those found in previous studies. In general, effects of subliminal semantic priming can be fragile and are difficult to replicate (60). Hence, differences in study design may profoundly affect the size of the effect as is applicable to the current findings and may possibly explain why we did not observe significant changes in MAP and HRV. First, Garfinkel et al. (2016, 61) and Hull et al. (2002, 62) used the words 'angry' and 'relax', whereas Levy et al. (2000, 198) used negative and positive stereotypes of aging. In the current study, general (i.e., nonspecific) threatening words were used. So, the type of words (in terms of specificity and relevance) may affect the physiological responses differently. This could result in for example stronger physiological responses to subliminally presented "bombardments" of the single word 'angry' (62) or to words that are particularly relevant to the study population (198). Second, some important other differences in study design may have affected the results, such as the use of a within-subject design with alternating words categories ( $r_s > .50$ ; 61) instead of between-subjects presentation of words with the similar valence and the use of single measurements at the end of experimental phases (62) rather than continuous measurements. Finally, despite the discrepancies in findings regarding BP and HR activity to subliminal primes, the absence of changes in HRV were in line with previous studies (61). Overall, the smaller, but practically meaningful, effects found in the current study are likely to be

attributed to specific design features of the different studies described and warrant exact replications of the previous studies.

Furthermore, the small changes of MAP and HRV in response to subliminal threat can also be explained in light of the work of other research groups with different priming methods that found larger changes in preejection period and systolic BP activity after stress-related subliminal priming (e.g., 132,144,163). In those studies, the effect of a prime was inferred from responses during a task that required a certain effort, such as a mental concentration task (163). Gendolla and colleagues explain their findings in the light of the effort-related cardiac response. The primes are regarded as an additional source of difficulty that increase CV responses when performing a challenging task. Sad and angry primes are found to elicit more effort, expressed in CV activity, than happy primes (e.g., 163). This implies that negative affective subliminal stimuli further augment CV responses when presented during a challenging task. Regarding the role of unconscious stress in daily life, this would imply that encountering threatening information outside of awareness elicits a small physiological response that is substantially stronger when this encounter concurs with a second strenuous event. Thus, although we consider subliminal semantic priming a formally suitable method to test the effect of unconscious stress on CV responses, other methods such as providing a challenging task during priming should also be considered.

We did not find a moderating role of trait worry in the effect of condition on TPR and HRV activity and a small effect on MAP activity. As discussed above, we presented general threat words of which we expected that, especially in high level trait worriers, would increase physiological responses. It could be that the threatening words, despite being validated for valence, were perhaps not sufficiently related to threat as experienced by our sample. In the study by Levy and colleagues (2000, 198), the prime words related to stereotypes of aging were validated in both an older and younger population (244) and were presented to participants older than 60. Importantly, Öhman and Soares (1994, 245) found that individuals with spider phobia showed more skin conductance responses to subliminally and supraliminally presented pictures of spiders compared with other (neutral) stimuli and that, similarly, those with snake phobia showed more skin conductance responses to pictures of snakes than compared with other (neutral) stimuli. Furthermore, it has been found that, in worriers, cognitive control (i.e., switching between different stimulus types) was impaired only when the negative stimuli were personally relevant to the participants (246). Thus, in the current study the threatening nature of the words might not have related closely enough to the worries of a student sample to elicit large CV changes.

Likewise, we did not find a moderating role of resting HRV in the effect of condition on CV activity. However, it should be noted that, irrespective of condition, higher resting HRV was related to larger increases in MAP and larger decreases in HRV reactivity. This is consistent with findings that higher levels of resting HRV are

commonly related to higher CV reactivity, which is suggestive of flexible responses to changing environments (223-227).

Furthermore, exploratory analysis indicated a possible role of gender and implicit affect regarding the effect of subliminal primes on changes in TPR. More specifically, females displayed similar TPR reactivity to threatening and neutral words, but males displayed lower TPR reactivity to neutral words. However, the current study was not designed to explicitly address the role of gender. Therefore, we recommend further research on gender differences in CV responses to stress and subliminal priming in general. Additionally, indirectly measured positive affect after threat or neutral priming was found to be related to changes in MAP. This indicates that subliminal priming might influence affect measured indirectly (i.e., not assessed through self-report) and warrants further research on these types of measures in unfolding the relationship between stress and CV disease. Although statistically the results were only marginal significant, they could hold important implications for further development of the concept of unconscious stress.

Above, we already mentioned some limitations, but one additional limitation of the current study is that, due to technological constraints, we were unable to measure concrete stimulus- or event-related responses to the primes. This is in contrast to, for example, the studies by Garfinkel and colleagues (2016, 61) who studied the direct response to each specific prime word. Using event-related responses would have allowed for inferences on development of the CV activity throughout the priming task. Perhaps most of the CV changes occurred at the beginning of the priming task. It also limits interpretation of effects of specific words or word categories and of differences between primes and targets regarding CV activity. The use of event-related responses in future studies is recommended. Furthermore, we have not included a condition with positive primes. Positive stimuli have been shown to elicit an affective and physiological response relative to neutral stimuli (e.g., 133,149). However, we designed the study to test the effect of an induced threatened state relative to an induced neutral state as this most closely related to our main research question in which we focus on unconscious stress rather than on unconscious happiness. Additionally, adding a third group to the design would have required a larger sample, which was not feasible within the given parameters in which the study was conducted. Given that a larger sample size would have been required where it would not answer our research question, we have not included a positive condition, but considering the potential theoretical relevance, we would encourage researchers to do so in the future. Finally, a slower RT in response to threatening primes would be expected (e.g., 61). We did find a small, but statistically nonsignificant, difference in RT between threat and neutral primes. However, with the current between-subjects design, it was not possible to draw any firm conclusions on the extent of encoding of the primes as behavioral comparisons between prime type was not possible. Thus, future studies

should include within-subject information on the behavioral differences in response to threat or neutral stimuli.

This is the first study to explicitly address the role of processes outside of awareness in the relation between stress and health, or unconscious stress, by using a subliminal semantic priming paradigm. By subliminally presenting threatening or neutral words to a healthy population, we expected to find larger CV responses to the threatening words, especially in individuals that are known to be at risk for CV health problems. We found a higher TPR of small effect size that was statistically significant, and small meaningful effect sizes on MAP and HRV that were not statistically significant, in response to the subtle threat cues. Further research is needed to clarify the role of unconscious stress in such a way that it is more closely related to the concept of stress in daily life.



