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

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Chapter 4

Subliminal anger and relax primes show similar cardiovascular activity patterns

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Abstract

Stress-related stimuli may be presented outside of awareness and may ultimately influence health by causing repetitive increases in physiological parameters, such as blood pressure (BP). In this study, we aimed to corroborate previous studies that demonstrated BP effects of subliminally presented stress-related stimuli. This would add evidence to the hypothesis that unconscious manifestations of stress can affect somatic health. Additionally, we suggest that these findings may be extended by measuring affective changes relating to these physiological changes, using measures for self-reported and implicit positive and negative affectivity. We presented either the prime word 'angry' ($n = 26$) or 'relax' ($n = 28$) subliminally (17 ms) for 100 trials to a student sample and measured systolic and diastolic BP, heart rate (HR) and affect. The 'angry' prime, compared to the 'relax' prime, did not affect any of the outcome variables. During the priming task, a higher level of implicit negative affect was associated with a lower systolic BP and diastolic BP. No association was found with HR. Self-reported affect and implicit positive affect were not related to cardiovascular (CV) activity. In sum, anger and relax primes elicited similar CV activity patterns, but implicit measures of affect may provide a new method to examine the relationship between (unconscious) stress and health.

The idea that the *unconscious* can influence the physiological state, as proposed more than a century ago, was replaced in the mid-20th century by a body-mind perspective that was more strictly based on observable behavior (for a review see 48). The last two decades have seen a swiftly growing new interest in unconscious (i.e., implicit) affectivity, and more recently in its relevance to health (26-28). It has been proposed that people are not aware of part of their cognitive-affective states induced by stressful events, while this may still influence their physiology to the extent that it may threaten their health (26,27). Most studies reporting on the relationship between negative cognitive-affective states, including worry, and prolonged physiological activity still rely only on self-report (see for example reviews by 15,21), despite studies suggesting that changes in physiological states often do not relate to what is reported (e.g., 247). Furthermore, physiological activation during sleep, when one cannot actively engage in cognitive processing, has been found to relate to stressors that occurred during the day, but not to self-reported affectivity (e.g., 15,22,38). Finally, subliminal negative affective stimuli (i.e., those presented below the awareness threshold) have repeatedly been shown to increase activity in the amygdala and other parts of the 'emotional brain', startle responses, and skin conductance (see reviews by 26-28,203). Taken together, this suggests that the relationship between psychological stress and health may be further explained by negative affectivity beyond self-report. Experimental evidence for this view would be a demonstration that stress-related, or negative affective, stimuli presented *outside of awareness* can increase health-relevant physiological responses, and that this increase is due to affective responses measured at different levels of awareness.

In a recent systematic review, we evaluated the effects of negative affective stimuli presented below the threshold of awareness (i.e., subliminally) on peripheral health-related physiological activity (203). Subliminal negative affective stimuli compared with non-affective stimuli were found to increase systolic blood pressure (SBP). Similar, but less consistent, results were found for other outcomes such as diastolic blood pressure (DBP) and heart rate (HR), which suggest that what is presented outside of awareness may have consequences for one's health. Additionally, in an experimental study we found that in response to subliminally presented threatening words, compared to neutral words, mean arterial pressure and total peripheral resistance (TPR) increased and heart rate variability (HRV) decreased (248). These studies indicate that a presentation of negative affective stimuli outside of awareness results in health-relevant vascular changes, but, as we indicated in the review (248), the number of studies for each CV outcome measure is limited, which warrants further research. Moreover, the promising and novel findings from the experimental study and the inconclusive results from the systematic review call for a replication of existing studies to confirm their findings and accumulate evidence for the effect of 'unconscious stress' on physiology, in line

with the contemporary emphasis on the need for replication in the social sciences (e.g., 249).

In terms of relevance to health, the most important studies using subliminally presented stress-related stimuli are those that have targeted health-relevant physiological parameters such as blood pressure (BP). In two studies, by Garfinkel et al. (2016, 61) and Hull, Slone, Meteyer, and Matthews (2002, 62), a larger SBP response to the subliminally presented word 'angry' was observed, when compared with the response to the subliminally presented word 'relax'. In addition to changes in SBP, changes in DBP were found in study 3 from Hull et al. (2002, 62) and changes in HR were found in study 4 from Hull et al. (2002, 62). Garfinkel et al. (2016, 61) did not find changes in HR or HRV. While Hull et al. (2002, study 3 and 4, 62) used a between-subjects design and presented the two primes for 100 trials in two separate conditions, Garfinkel et al. (2016, 61) used a within-subject design and presented the two primes in 200 trials divided in blocks of four (study 1) and six (study 2) trials while recording fMRI in addition to the CV variables. These very similar studies seem to indicate that repetitive presentation of negative affective stimuli induces changes in peripheral physiological parameters. Therefore, we aimed to contribute to the body of knowledge by once again testing whether subliminally presenting the word 'angry' would lead to a larger CV response when compared to the word 'relax' (Garfinkel et al. (2016, 61) and Hull et al. (2002, 62).

In the current study, in addition to verifying these previous findings, we implemented several methodological improvements. In the study by Hull et al. (2002) the BP measures were taken with single arm cuff measures which are less reliable than continuous measures (250), and the experimenters were not blind to the priming condition. The study by Garfinkel et al. (2016, 61) was partially based on the study by Hull et al. (2002, 62), but focused mainly on the combined effect of the supposedly induced affective state and physiological responses on cognitive processing. Importantly, Garfinkel et al. (2016, 61) used a within-subject design (where a between-subjects design was used in the original studies), measured BP continuously throughout the experiment, and the experimenters were blind to the conditions due to computerized randomization and stimulus presentation. Furthermore, the within-subject approach facilitated the fMRI testing procedures required to address the authors' neurobiological research questions. Moreover, in Garfinkel et al. (2016, 61) the possible carry-over effects due to the within-subject procedure cannot be ruled out. Thus, the between-subjects design, similar to Hull et al. (2002, 62) is preferred. The main difference with the previous studies was that we used a double blind design and continuous BP measures.

The case for a CV effect of unconscious stress would become stronger if we could additionally show that changes in CV activity to subliminal primes are mediated by affective responses measured at different levels of awareness. Therefore, changes in the affective state were assessed to corroborate the findings on the physiological

parameters. Additionally, in the study by Hull et al. (2002, 62) the Positive and Negative Affect Scale (254) was used to assess affect. Notably, Van der Ploeg, Brosschot, Thayer, & Verkuil (2016, 202) and Brosschot et al. (2014, 88) have shown that in addition to self-reported affect, affective processing at an implicit level is related to CV responses to a stressor. For this purpose measures that assess affect indirectly can be used, such as the Implicit Positive And Negative Affect Test (IPANAT; 83,84,252). The IPANAT is designed to measure automatic activation of cognitive representations of affective experiences (84). It takes advantage of the process of affect infusion (86) by asking people to rate the extent to which nonsense words are indicative of certain emotions. It is suggested that the ratings are indicators of automatic activations of their negative or positive affective representations. Furthermore, low implicit positive affect (IPA) predicted circadian cortisol release, and implicit negative affect (INA) predicted greater cortisol responses to acute stress, whereas again no link between self-reported affect and cortisol was found (85,87). Notably, there is also evidence that high IPA (rather than low INA) is related to the effective regulation of threat and stress (253,254). The absence of relationships between self-reported affect and physiological outcomes indicates that merely assessing self-reported affectivity is insufficient. Moreover, in reality both self-reported and implicitly measured affect are highly likely to co-occur (66). Thus, in addition to the replication of the mentioned studies, we aimed to assess the mediating role of self-reported affect, INA, and IPA in CV reactivity.

In the present study we attempted to show that subliminal negative affective stimuli can increase CV activity relating to the findings of Garfinkel et al. (2016, 61) and Hull et al. (2002, 62), and to test whether this effect is due to changes in negative affect outside of awareness. More precisely, we expected that repeated subliminal priming with the word 'angry' as opposed to the word 'relax', would increase SBP, DBP, and HR. In addition, we expected that this increase would – at least partly – be mediated by increased INA and/or IPA, with implicit anger in particular, as measured with the IPANAT, and self-reported negative and positive affect, with self-reported anger in particular. Together, the findings will clarify the role of unconscious processes in stress-related CV activity.

Method

Participants

Students from Leiden University could sign up for the experiment and received four euro or course credits for their participation. They provided informed consent before the start of the experiment. Participants were randomly allocated to the angry prime ($n = 26$) and relax prime ($n = 28$) condition through a computerized procedure to which the experimenter was blind. The experiment was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University.

Instruments

Cardiovascular activity

The CV measures were recorded continuously during the experiment using the Portapress Model-2 (Finapres Medical Systems, Amsterdam, The Netherlands). Using this non-invasive method, BP was measured through a finger cuff that was placed on the middle finger of the non-dominant hand. The signal was visually inspected and manually corrected for artefacts in Acqknowledge 3.9.1.4. SBP and DBP (in mmHg), and HR (in bpm) were derived from the signal using a tailor made toolbox in Matlab R2012b. The average CV activity during five measurement periods (acclimatization, baseline, practice, prime, recovery) was calculated for the outcome measures. TPR (mmHg.min/L) was calculated using BP and HR (229,230) to corroborate previous findings (248).

Questionnaires

Self-reported levels of affect were assessed with a visual analogue scale (VAS) on which participants had to indicate to which extent they felt a certain emotion (happy, scared, sad, joyful, gloomy, angry, fear, annoyed) on a scale from zero to 100. The subscales, explicit negative affect (ENA) and explicit positive affect (EPA), were reliable with a Cronbach's alpha of .71 and .69, respectively. Considering that we aimed to manipulate angry affectivity, we also extracted the anger subscale, which was sufficiently reliable with a Cronbach's alpha of .69.

To assess affect at an implicit level the IPANAT (the Dutch version used in 202, study 2) was provided, using five nonsense words (vikes, tunba, ronpe, belni, sukov) that were rated on 12 emotional adjectives (sad, gloomy, unhappy, annoyed, irritated, angry, afraid, frightened, scared, joyful, cheerful, happy) using a six-point Likert scale. The reliability of the IPA and INA subscales were Cronbach's alpha .52 and .87, respectively. The word TUNBA negatively affected the reliability of the PA scale and the relating items were omitted for the analysis resulting in a Cronbach's alpha of .65 (PA) and .87 (NA). This can be considered sufficient but compared with previous studies the reliability of the PA subscale was somewhat low (84,88,202). Similarly, the Cronbach's alpha of the anger subscale, without the TUNBA items, was .65, which is also sufficient.

Subliminal priming task

The subliminal priming task was based on Hull et al. (2002, 62). During a Lexical Decision-making Task (LDT) using Dutch words, participants were asked to determine as fast as they could whether the target was a word (e.g., "cursief", "concept") or a nonword (e.g., "toncepc", "lardboa") by pressing 2 or 8 on the numerical pad of the keyboard. The words were selected from a list of a hundred seven-letter nouns that have shown low emotional associations (255). The nonwords were derived from the words by replacing the vowels with another vowel and consonants with another

consonant. Ten words and 10 nonwords were randomly chosen from two lists for practice trials, whereas 50 words and 50 nonwords were randomly selected and presented during the experimental trials. No feedback was provided on accuracy or speed of the responses.

The targets were preceded by a fixation cross (500 ms), a forward mask ("IDXFNBO", 17 ms), the prime word (17 ms), a blank screen (17 ms), a backward mask ("IDXFNBO", 50 ms), and a blank screen (100 ms). The target presentation ended upon responding. During an initial set of 20 practice trials a neutral prime word (the Dutch 'neutraal') was shown. In the 100 experimental trials the prime words 'woedend' [angry] or 'rustig' [relaxed] were presented depending on condition. These two primes were chosen from an array of several potential translations of the original English primes based on a small pilot study with 15 individuals, who did not participate in the final study. We presented eight different Dutch words thought to represent angry and six for relax. The participants rated the degree to which these words would have the same emotional impact as the English words on a scale from 1 to 10. The two words with the highest score and the lowest inter-rater variance were selected. The task was presented on a CRT monitor with a resolution of 800 x 600 pixels and a refresh rate of 75 Hz. The experiment was executed using E-Prime 2.0.8.90.

Behavioral data consisted of the reaction times (RT) to the targets. RTs faster than 100 ms and slower than 1500 ms, incorrect responses (5.59%), 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 ($n = 1$ of the final sample). Mean RTs were calculated across trials and for word and nonword trials separately.

To determine whether the 17 ms stimulus presentation of the subliminal prime was short enough to prevent conscious recognition of the words, an awareness check was provided at the end of the experiment (121). We provided a forced choice prime recognition (AFC) task and subliminally presented, similar to the experimental phase, five 'angry' and five 'neutral' prime words. After each trial, participants had to indicate what word they believed to have seen. To assess sensitivity, the proportion of correct responses was calculated (256). Additionally, participants indicated how well they could see the image ("I could clearly see the word", "I saw something, but I did not see the word", or "I did not see anything") to indicate their experienced level of clarity. Participants that scored high on sensitivity and clarity, providing information about subjective and objective awareness, were assumed to have consciously perceived the primes (121,122).

Procedure

After providing informed consent, participants were attached to the recording apparatus while seated facing the monitor. The subjects were randomly assigned to

either the experimental ('angry') or control ('relax') condition. Participants were then instructed to relax and clear their mind for five min to get used to the instruments (acclimatization phase). Before continuing with the experiment, participants filled out a questionnaire on demographics and biobehavioral factors. They were told that they would be working on a "decision task." A baseline measurement was performed for three min (baseline phase), which was followed by the practice phase and the experimental phase (prime phase). Immediately after the priming task, participants completed the IPANAT, the VAS, and the awareness check, which was followed by a period of relaxation for five min (recovery phase). Finally, participants were carefully debriefed. The experiment took about 45 min.

Statistical analyses

After data inspection, independent *t* tests or chi-square tests, depending on measurement level of the variables, were used to explore potential differences between the two conditions ('angry' vs. 'relax') on demographics, biobehavioral factors, and baseline CV measures. Differences between conditions in self-reported and implicitly measured affect and RT were analyzed using one-sided independent *t* tests, as we expected higher SBP, DBP, and HR in the 'angry' condition compared to the 'neutral' condition. Repeated-measures analyses of variance (RM-ANOVA) were used to test the effects of the between-subjects factor Condition ('angry' vs. 'relax') on the CV variables across the three experimental phases, that is, factor Time (baseline, priming, recovery). Furthermore, the possible mediation of the CV effects by self-reported and implicitly measured affectivity were tested using parallel mediation analyses (240) on the change scores of the CV variables during the task and recovery (238). All analyses were performed with SPSS 23.0.

Results

Descriptive statistics

Out of the 74 participants that were tested, 54 cases were retained for the analyses (age: $M = 20.2$, $SD = 1.72$; 74.1 % female). Four participants were excluded because they had high BP or other medical conditions or current psychological health problems. Two participants used medication that may affect the ability to concentrate. The other 14 cases could not be used due to equipment failure. From these exclusions, 12 cases had been assigned to the 'angry' condition and eight to the 'relax' condition. The data were considered to be missing at random. The demographical information of the participants is provided in Table 1. No differences between conditions on baseline values of the demographics, biobehavioral variables, and CV measures were found. The sample consisted of mostly Dutch participants ($n = 48$, 88.6%), but all participants had a sufficient understanding of the Dutch language. Analyses were

performed with and without nonnative speakers and since the findings were similar, those with both native and nonnative speakers are reported.

Participants reported not to have seen the subliminal stimuli ($M = 1.65$, $SD = 0.37$) in the awareness check, suggesting that the subliminal presentation of the stimuli had been successful (121). However, the results from the AFC indicated that two participants correctly identified 75% of the images in the awareness check. Although they did not report to have seen the images, one of them correctly identified 68.8 % and had a mean clarity of 2.50, which is high considering the maximum score of 3. This combination of objective and subjective reported awareness provides sufficient reason to assume that this participant was aware of the stimuli (256). Analyses were performed with and without this participant, which did not result in meaningful differences, and those including this participant are reported.

TABLE 1 Baseline characteristics stratified by condition

Measure	Total (<i>N</i> = 54)		Angry (<i>n</i> = 26)		Relax (<i>n</i> = 28)		<i>t</i> / χ^2
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
<i>Demographics</i>							
Age, years	20.2	1.72	20.0	1.50	20.3	1.92	-0.61
Female sex ^a	40	(74)	21	(81)	19	(68)	1.17
BMI	22.2	2.71	22.4	3.17	22.0	2.25	0.49
Dutch nationality ^a	48	(89)	22	(85)	26	(93)	-1.17
<i>Biobehavioral variables</i>							
Smoking ^a	5	(9)	3	(12)	2	(7)	0.31
Drugs ^a	7	(13)	4	(15)	3	(11)	0.26
Caffeine use (average glass/day)	1.35	0.63	1.42	0.61	1.28	0.67	0.68
Alcohol use (>5 glasses day/month)	2.09	2.44	2.52	2.81	1.71	2.04	1.16
Visits GP (last 6 months)	0.98	1.22	0.92	1.06	1.04	1.37	-0.34
<i>Cardiovascular measures</i>							
SBP	123.4	16.0	123.5	17.9	123.3	14.4	0.41
DBP	66.9	11.5	67.0	13.3	66.9	9.87	0.44
HR	77.9	12.2	77.7	11.8	78.0	12.8	-0.09
TPR ^b	4.47	0.24	4.45	0.27	4.49	0.20	0.54

Note. Abbreviations: BMI = Body mass index, GP = General practitioner, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, TPR = Total peripheral resistance. The cell sizes are displayed as the amount of usable recordings varied across outcome measures. TPR was square root transformed. All tests were performed two-sided. There were no significant differences between the conditions.

^a Indicated with number of positive responses (percentage), Pearson χ^2 was used as test statistic.

^b In both conditions one participant was excluded

Task performance

The overall mean RT in the angry prime condition ($M = 766.8$, $SD = 123.0$) did not differ from the relax prime condition ($M = 735.3$, $SD = 102.3$, $t(51) = 1.01$, $p = .32$, $r = .140$). Similarly, there were no differences in RT to the nonwords between the angry prime ($M = 749.6$, $SD = 126.4$) and relax prime condition ($M = 761.8$, $SD = 109.7$, $t(51) = 0.38$, $p = .71$, $r = .052$). However, in the angry prime condition a slower RT was found ($M = 784.0$, $SD = 125.4$) in response to words compared with the relax prime condition ($M = 708.3$, $SD = 98.4$, $t(51) = 2.46$, $p = .017$, $r = .33$).

Affect

To examine the effect of the priming condition on affect, independent t tests were performed on the subscales of the affect measures, one-sided (e.g., 257). Self-reported NA and anger were not normally distributed and tested non-parametrically. In terms of expected effects, participants in the angry prime condition displayed statistically non-significant higher INA, ($t(52) = 1.19$, $p = .24$, $r = .36$), self-reported NA ($U = 289$, $z = 1.30$, $p = .19$, $r = .18$), and lower self-reported PA ($t(52) = -1.25$, $p = .22$, $r = .17$) compared with the relax prime condition. Specific tests on the anger subscales showed that self-reported anger in the 'angry' condition did not differ from the 'relax' condition ($U = 399.5$, $z = 0.43$, $p = .67$, $r = .059$). In contrast, implicitly measured anger was higher in the 'angry' condition ($M = 3.54$, $SD = 0.63$) compared with the 'relax' condition ($M = 3.16$, $SD = 0.68$, $t(52) = 2.11$, $p = .039$, $r = .28$). No meaningful differences were found for IPA, $t(52) = 0.029$, $p = .98$, $r = .004$. Results are displayed in Table 2.

TABLE 2 Affect ratings after priming stratified by condition

Measure	Angry ($n = 26$)		Relax ($n = 28$)		t	r
	M	SD	M	SD		
<i>Implicit affect</i>						
NA	3.06	0.58	2.86	0.63	1.19	.36
Anger	3.54	0.63	3.16	0.68	2.11**	.28
PA	3.12	0.69	3.11	0.63	0.029	.004
<i>Self-reported affect</i>						
NA ^a	7.63	-	6.66	-	1.30	.18
Anger ^a	3.33	-	1.60	-	0.43	.059
PA	21.1	27.3	29.9	24.3	-1.25	.17

Note. All t tests were performed one-sided. *Abbreviations:* NA = Negative affect, PA = Positive affect.

^a Mann-Whitney U Z statistic, with Medians (Md).

* $p < .10$, ** $p < .05$, *** $p < .01$

Cardiovascular activity

A two-way RM-ANOVA to assess the impact of condition on the CV variables across the three experimental phases was performed for each outcome variable. The results are displayed in Table 3. One participant (in the 'relax' condition) showed deviating HR responses throughout out the experiment (i.e., a bpm of around 110). Analyses with and without this participant led to meaningful differences, that is, the overall mean was substantially higher during priming ($\Delta M = 1.06$) and the recovery ($\Delta M = 0.99$) when this participant was included. Results without this participant are reported to be conservative. For SBP, there was no significant Time \times Condition interaction ($F(2,50) = 0.38, p = .69, = .015$). There was an effect of Time ($F(2,50) = 24.2, p < .001, = .49$), but not of Condition ($F(1,51) = 0.001, p = .98, < .001$). For DBP, there was no significant Time \times Condition interaction ($F(2,50) = 0.46, p = .63, = .018$). Similarly, there was an effect of Time ($F(2,50) = 15.1, p < .001, = .38$), but not of Condition ($F(1,51) = 0.008, p = .93, < .001$). Furthermore, for HR there was no significant Time \times Condition interaction ($F(2,49) = 0.57, p = .57, = .023$), neither an effect of Time ($F(2,49) = 2.39, p = .10, = .089$), nor of Condition ($F(1,50) = 0.032, p = .86, = .001$). Finally, no significant effects were found for TPR with $ps > .25$ and $s < .03$. Figure 1 displays the BP and HR activity during baseline, the priming, and the recovery.

The mediation analyses revealed that INA, but not IPA, was associated with changes in SBP and DBP during the priming task ($b = -4.02, SE = 1.97, t(52) = -2.04, p = .046, r = .27$ and $b = -3.24, SE = 0.79, t(52) = -4.11, p < .001, r = .50$, respectively). During the recovery, this association was statistically not significant anymore, for DBP ($b = -1.89, SE = 0.96, t(51) = -1.97, p = .055, r = .27$) nor SBP ($b = -0.89, SE = 2.02, t(51) = -0.44, p = .66, r = .061$). Analyses with the anger and PA subscales revealed an association of implicitly measured anger with changes in DBP, but not SBP, during the task ($b =$

TABLE 3 Descriptive statistics and test statistics of the cardiovascular variables stratified by condition and the two main experimental phases, during the task and recovery

Outcome measure	Task				Recovery				F'	df	
	Angry		Relax		Angry		Relax				
	M	SD	M	SD	M	SD	M	SD			
SBP	130.2	17.7	129.7	17.2	129.7	19.5	128.8	15.1	0.38	2, 50	.015
DBP	68.9	12.7	69.5	10.3	68.9	13.2	68.5	10.6	0.63	2, 50	.018
HR ²	77.5	10.3	76.5	11.6	76.3	10.7	76.3	10.4	0.57	2, 49	.023
TPR ³	4.41	0.24	4.49	0.25	4.44	0.26	4.47	0.24	1.25	2, 48	.049

Note. Abbreviations: SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, TPR = Total peripheral resistance.

¹ Results from the RM-ANOVA Time \times Condition, where Time is Baseline, Task, and Recovery and Condition is Angry or Relax.

² One participant was excluded in the 'relax' condition.

³ In both conditions one participant was excluded

-2.13, $SE = 0.80$, $t(52) = -2.68$, $p = .010$, $r = .35$), but not statistically significant during the recovery ($b = -1.58$, $SE = 0.91$, $t(51) = -1.74$, $p = .088$, $r = .24$). No associations were found with changes in HR. Furthermore, TPR during the task was negatively associated with IPA ($b = -0.07$, $SE = 0.03$, $t(46) = -2.12$, $p = .039$, $r = .30$), TPR during the recovery was also negatively associated with IPA ($b = -0.07$, $SE = 0.03$, $t(46) = -2.24$, $p = .030$, $r = .31$) and (statistically marginally significant) with INA ($b = -0.06$, $SE = 0.03$, $t(46) = -1.89$, $p = .066$, $r = .27$). Moreover, explicit negative and positive affect were not statistically significantly associated with CV activity ($ps > .10$).

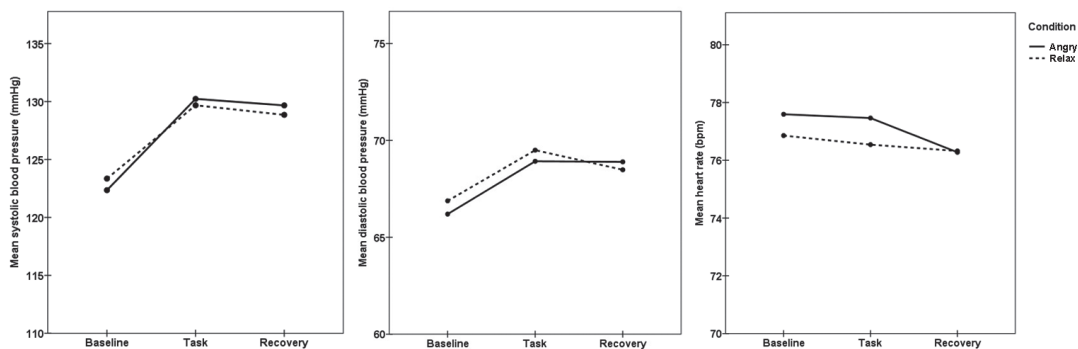


FIGURE 1 Cardiovascular activity throughout the experiment displayed per condition for systolic and diastolic blood pressure and heart rate

Discussion

The idea that part of the health-related physiological stress responses may be due to stress-related cognition outside of awareness seems to be supported by studies showing that subliminal negative affective stimuli, compared to neutral stimuli, induce changes in CV responses. In the current study, we aimed to verify some of these findings. Subliminal presentation of the word ‘woedend’ [angry], compared to the word ‘rustig’ [relax], was expected to increase SBP, DBP, and HR, in line with the studies by Garfinkel et al. (2016, 61) and Hull et al. (2002, 62), and this effect was thought to be mediated by self-reported and implicit measures of affect. In the current study, the subliminal negative affective stimuli did not elicit a higher SBP, DBP, or HR. Finally, we found an association of changes in SBP and DBP during the task with INA, but not with self-reported affect or IPA. However, these significant associations were not in the hypothesized direction, that is, larger increases in BP were associated with lower levels of INA. Additionally, HR was not related to the measures of affect.

With respect to the earlier studies, we have not found differences in SBP and DBP to either an ‘angry’ or a ‘relax’ prime. With the current repeated-measures design,

a sample size of 44 was sufficient to detect a small effect, with a power of 90% (G*Power 3.1). It therefore seems unlikely that the current sample was too small and the study was at risk of a Type II error (see for example 258). The effect sizes were small and the plots also did not clearly indicate any effect of the priming procedure on CV activity. As discussed previously, the differences from the study of Garfinkel et al. (2016, 61) may partly explain the discrepancy in findings between studies. The within-subject design increases the power of the study by Garfinkel et al. (2016, 61) and may be one important factor. With respect to the study by Hull et al. (2002, 62), the use of intermittent measures of BP may have led to a transient assessment of the vascular changes that does not necessarily represent the overall changes in BP.

However, another, and perhaps more likely, explanation of the current findings could be the Dutch translation used for the word 'angry' [woedend]. Both Garfinkel et al. (2016, 61) and Hull et al. (2002, 62) used the English word 'angry', which may be used quite often in the English language. In contrast, 'woedend' is not used that often in Dutch and perhaps even less so amongst college students. Additionally, although it is the most adequate translation, it may not be ascribed the same affective value as 'angry'. Another primary emotion-word that is more commonly used in Dutch is 'boos'. From a database using subtitles to indicate the frequency of Dutch per million words, the found frequency of 'woedend' is 8.35 and that of 'boos' is 105.79 (259). Although for this study we used a careful selection method of the prime words, the raters in this procedure may have focused too much on an adequate translation rather than affective value and, as a consequence, 'woedend' did not elicit a sufficient level of arousal to generate an effect (260). On the other hand, we did observe a significant increase in implicitly measured anger in the angry condition, although this could have been largely a semantic effect. Thus, several methodological differences may account for the absence of an effect of the primes on SBP and DBP.

Some additional findings require a brief discussion. During the priming task and during the recovery from this task SBP and DBP, but not HR, were higher compared to the levels at baseline, irrespective of condition. This suggests that the LDT, during which the priming took place, increased mental task demands (261) and induced task engagement (100) independent from type of prime words. As Sosnowski et al. (2010, 261) indicate, RT based tasks appear to elicit larger changes in BP indices but not in HR. Only in study 4 by Hull et al. (2002, 62) an effect of prime type was found for HR, but not in the current nor the other studies by Hull et al. (2002, 62) and Garfinkel et al. (2016, 61). Thus, although the CV changes did not differ between conditions, the observed pattern in response to performing a LDT is congruent with previous findings.

The primes were not associated with any statistically significant changes in affect, but some small effect sizes were found that are theoretically relevant, as they were in the predicted direction. More specifically, self-reported NA increased and self-reported PA decreased in the 'angry' prime condition. Additionally, the increases in INA, considering

the subtle manipulation, are noteworthy despite their statistical non-significance. A possible reason for the absence of statistical evidence for a role of affect in this study may be the lack of baseline measurements of the affect measures. However, this was done intentionally to prevent any carry-over effects of the presentation of affective words before the priming procedure. Moreover, we cannot exclude effects of pre-existing affective states on the outcomes. Future studies should aim to assess both self-report and implicit measures of affect at baseline. Furthermore, relative to previous research, as described in the Method, the reliability of the IPANAT was low which should be kept in mind when considering these results.

Importantly, a specific difference between conditions on the implicit anger scale was apparent and may indicate an emotion-specific effect of the 'angry' prime on implicit affect, which could have been averaged-out by looking into the more general INA subscale. Notably, we did find associations of INA with SBP and DBP during the task, irrespective of condition. These associations seem to indicate that when one is high in implicitly measured negative affect or anger in particular, BP is lower during a task, which is not considered to be an adaptive response. Additionally, a negative relationship between TPR and IPA was found. TPR is thought to indicate physiological responding to challenge or threat (99,100). The current finding suggests that a higher IPA is related to experiencing the task as challenging, rather than threatening, which is compatible with previous findings of a relationship between IPA (but not INA) with effortless affect regulation (e.g., 253,254), which helps individuals to put negative experiences in perspective and regard them as challenges (e.g., 262). In general, these findings highlight the additional value of measures of affect beyond self-report, which were not related to changes in CV activity. Taken together, although there was a role for measures of affect at an implicit level, changes in affect beyond self-report do not seem to be instigated by subliminal priming and may become evident in sufficiently intense stressful situations.

To summarize, in the current study we have aimed to verify results from previous studies that found increased CV activity in response to the subliminally presented word 'angry' vs. 'relax'. Unfortunately, we did not find effects of subliminal priming with the word 'angry' on cardiovascular activity as support for the unconscious stress hypothesis. Still, the findings indicate that new additional measures, the IPANAT and TPR, may contribute to a better understanding the role of unconscious processes in the physiological effects of psychological stress.

