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

Ploeg, M.M. van der

Chapter 5

Subliminal and supraliminal fear conditioned stimuli increase electrodermal but not cardiovascular responses

Melanie M. van der Ploeg, Jos F. Brosschot, Bram Vervliet, Omer Van den Bergh, and Bart Verkuil

Published as:

Van der Ploeg, M. M., Brosschot, J. F., Vervliet, B., Van den Bergh, O., & Verkuil, B. (2018) Inducing unconscious stress: Subliminal and supraliminal fear conditioned stimuli increase electrodermal but not cardiovascular responses (submitted).

Abstract

Stressors processed outside of awareness may activate physiological responses. This unconscious stress may result in adverse health outcomes such as cardiovascular (CV) disease. The current "proof of principle" study tested whether fear conditioned images, as operationalization of a stressor, would elicit physiological responses when presented subliminally. In the acquisition phase, students (N = 93) were exposed to a set of neutral images, of which two were paired with an electric shock (CS+) and two were not (CS-). The participants were explicitly informed on this association to ensure contingency awareness. In the test phase, they were randomly assigned to either subliminal (n = 41) or supraliminal (n = 52) presentation of the CS+ and CS-. Responses to the CS+, as compared to the CS-, were measured for skin conductance response (SCR) magnitude, systolic (SBP) and diastolic blood pressure (DBP), and heart rate (HR) in both the subliminal and the supraliminal group. The manipulation check indicated that SCR magnitude was indeed increased in response to the CS+ compared to the CS-, but this differential effect was not found for SBP, DBP, and HR. In the test phase, SCR magnitude, but not CV activity, increased in response to subliminal and supraliminal presentation of the CS+ compared with the CS-. These findings indicate that successfully fear conditioned images can elicit physiological responses when presented supraliminally as well as subliminally, but the expected effects were only apparent for electrodermal responses. Thus, only partial evidence for a physiological effect of unconscious stress was obtained.

Autonomic responses to psychological stress outside of awareness may contribute to the deterioration of health. Although many studies have documented that psychological stress is related to the development or worsening of cardiovascular (CV) disease (e.g., 2,3,5,6,9,115,201,263-265), the specific mechanisms underlying this relationship are still under debate (8,9). A stress response occurs when an organism is exposed to an aversive or threatening situation (i.e., a stressor) that may exceed available coping responses. This temporarily causes allostatic load which is accompanied by psychological, behavioral, and physiological changes that have to be reduced to return to homeostasis (16,118). However, when these changes become chronic, for example due to prolonged exposure to a stressor, the stress response is maladaptive and may lead to adverse health outcomes (10,11,13-16,115). Moreover, stressors activate negative affective cognitive processes, such as worry, that have been related to adverse physiological responses (e.g., 17,20,21,31-33). However, in studies that related psychological stress to CV responses, only a part of the CV activity could be explained by self-reported measures of stress, worry, or affect (e.g., 22-25,76,82). For example in an ambulatory study, Pieper et al. (2010, 25) found elevated CV activity, even when stressors and worries were no longer reported. Moreover, this elevated CV activity was also unrelated to negative affect or biobehavioral variables. These findings suggest that stress-related physiological activity may be affected by processes outside of awareness, here referred to as unconscious stress (26,27), which may at least partly explain the relationship between psychological stress and adverse health outcomes. We recently provided evidence for this hypothesis, by showing that prolonged CV responses to a laboratory stressor were partly explained by implicit positive and negative affect in addition to self-reported affect (202). Still, to date evidence is scarce.

The role of unconscious processes in physiological responding has previously been addressed in experimental neuroscience studies. Presenting fear-inducing stimuli below the threshold of awareness activates the amygdala (e.g., 41,45,46), which in turn triggers the autonomic nervous system and hypothalamic-pituitary axis (45). In other words, awareness of the stimuli is not necessary to activate affective information processing and peripheral physiological processes. However, despite ample studies using neuroimaging in this line of research, peripheral physiological responses have hardly been addressed, let alone peripheral responses that are health-relevant, such as CV responses. Only a few subliminal priming studies that presented stress-related stimuli have used these peripheral physiological parameters (e.g., 61,62; see for a review 203). These studies found an increase in systolic blood pressure (SBP) to negative affective stimuli compared with control stimuli. However, the findings for other CV parameters are diffuse. Additionally, a recent subliminal priming study of our own group with threatening versus neutral words (248) found an increase in total peripheral resistance, but no changes in other CV variables. Thus, based on the findings of priming research, subliminal presentation of stress-related stimuli may affect CV

activity, but the results are inconclusive. This may be explained by the nature of the stress-related stimuli used, which may not initiate a stress response in all participants in those studies. More specifically, the change in affect associated with the verbal and pictorial stress-related stimuli that are typically used may depend on individual learning histories. A solution to this would be to create stress-related stimuli by using fear conditioning, that is, temporarily create an association between an initially neutral stimulus and the stress response by using an individually determined aversive stimulus, and present these fear conditioned stimuli subliminally. Thus, demonstrating health-relevant physiological effects of fear conditioned stimuli of which people are not aware, or subliminal fear conditioned stimuli, would provide a proof of principle that unconscious stress can influence somatic health.

Fear conditioning initiates a nonspecific state of vigilance that is generally equated to the stress response (see for example 69,71), which is characterized by heightened attention and physiological activation (e.g., 70,72). This is in line with current learning theory-based stress theories (e.g., 16) and suggests that experimental fear conditioning can be used to, temporarily, create a stressor. Fear conditioning constitutes the automatic physiological response (i.e., unconditional response, UCR) to an aversive stimulus (unconditional stimulus, US), such as a shock. The repeated combined presentation of the US and a different stimulus during an acquisition phase results in a conditional response (CR) to the now conditional stimulus (CS+). As a result, the CR occurs even in absence of the original US, which is thought to represent a newly created association (CS-US). The existence of the CS-US association is often demonstrated in a differential conditioning paradigm, that is, by comparing the participants' response to the CS+ with a response to the CS-, where the CS- is a stimulus that was never paired with the US (e.g., 45,68). In a recent systematic review (203) no studies were found that measured the effect of subliminally presented fear conditioned stimuli on CV parameters. In the current study, to create personally relevant stress-related stimuli, we used a differential fear conditioning paradigm with initially neutral stimuli. Once the CS-US association (i.e., the stressor) was created, the stimuli were presented subliminally in a subsequent test phase, without the US, to examine the effect on peripheral physiological outcomes, including CV activity.

The use of initially neutral stimuli in fear conditioning is crucial to create personalized stressors of which the influence of pre-experimental learning histories is negligible. However, according to Mineka and Öhman (2002, 267) the effect of subliminally presented fear conditioned stimuli on autonomic responses would be limited to 'fear-relevant' stimuli, such as snakes or spiders, which intrinsically pose a threat to survival. In contrast, 'fear-irrelevant' stimuli, such as flowers and mushrooms, do not intrinsically pose a threat and therefore the physiological response to them would not or not easily be fear conditioned. This is referred to as the 'preparedness theory' (187,267,267). According to Mineka and Öhman (2002, 267) this difference

between stimuli is due to the absence of 'emotional learning' that accompanies fear-irrelevant stimuli, which would allow only for short-lived CS-US associations. However, two early studies have in fact successfully fear conditioned neutral stimuli and found an effect of subliminal presentation on skin conductance measures (111,128). Notably, in these studies the methods of subliminal presentation (i.e., using low level illumination; 128) and using a relatively long (75 ms) viewing time (111), are unconvincing methods to present stimuli outside of awareness in the light of modern day possibilities. Importantly, Mineka and Öhman (2002, 267) equate emotionally learned responses in fear conditioning to the expression of autonomic responses, but to our knowledge no studies have been conducted that assessed the effects of subliminal presentation of CS+ on CV variables in addition to skin conductance responses (SCR; 203). Therefore, in the context of subliminal presentation, the claim that emotional learning only occurs when fear-relevant stimuli are fear conditioned is not fully justified and remains to be explicitly tested. Summarizing, we expected that neutral images can be fear conditioned and henceforth can be used to test the influence of stress-related stimuli outside of awareness on SCR as well as CV variables.

In sum, previous findings suggest that subliminally presented fear conditioned stimuli affect SCRs, but this has not yet been tested for CV parameters. The current study was conducted in a healthy sample to test whether unconscious stress affects health-related physiology by using a fear conditioning paradigm with electrical shocks as the US. Neutral stimuli were supraliminally presented in the acquisition phase, followed by subliminal and supraliminal presentation in a test phase without the US. Subliminal presentation was achieved by displaying images for 20 ms followed by a mask using computerized presentation. Furthermore, objective accuracy of the presentation durations was tested beforehand to check whether the images were indeed displayed for such a short period. Additionally, participants performed an awareness check to validate the absence of stimulus awareness (121,122), Notably, to create contingency awareness we explicitly informed participants on the CS-US association (see 269). We expected that the participants would show a differential physiological response, that is, a larger SCR magnitude, BP, and heart rate (HR), to the CS+ during acquisition as a manipulation check. Crucially, we expected that without the presentation of the US this differentiation was retained in response to both subliminal and supraliminal presentation to represent physiological responses to ongoing stress-related cognitions of which one is either aware or not aware.

Method

Participants

We recruited a total of 128 students from Leiden University, The Netherlands, who received course credits or 7.50 euro for participation. Ten participants were excluded due

to current CV and/or psychological health problems, in accordance with our exclusion criteria. In eleven cases the experiment failed due to technical or experimenter error. In one case the participant had used soft drugs on the day of testing. Participants were rescheduled when they had drunk coffee or exercised within three hours prior to the experiment. Nonresponders, defined as participants showing no SCR to the US in the acquisition phase (as recommended, 269), were excluded from the data analysis (n = 13). The final sample of 93 participants had a mean age of 20.6 (SD = 2.73) and 68 were female (73.1%). Participants provided informed consent before the experiment. In the test phase participants were randomly assigned to the subliminal (n = 41) or supraliminal (n = 52) group, referring to the presentation method. The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University, under number 3964998062.

Apparatus and instruments

Stimuli. The CSs were four neutral images from the International Affective Picture System (numbers 7004 (spoon), 7052 (clothes pegs), 7090 (book), and 7595 (car); 270). The images were used in a 70 mm x 90 mm format and converted to greyscale. They were presented in the middle of the screen of a 100 Hz CRT monitor using a 800 x 600 pixels screen resolution against a grey background. From a wider selection of images tested in a pilot study these images were recognized least often when presented subliminally. As a mask a constellation of colored squares (85 mm x 110 mm) was presented. In the acquisition phase the stimuli were presented for 500 ms, as they were during the supraliminal trials in the test phase. During the subliminal trials, the images were presented for 20 ms, followed by a subsequent presentation of the mask for 500 ms. The interstimulus interval was 7 s. The actual duration of the stimulus presentation was checked before the start of the study using a light sensor test. The experiment was programmed in E-prime 2.0.8.90. The US was delivered using a shock stimulator (Grass, S48 Stimulator) with electrodes attached to the median nerve of the right wrist. Shock intensity was set at 150 Volts and 20 ms duration. The amount of current was set manually to a person-specific amount of maximally 15 mA through a US intensity calibration protocol (see procedure). Using these settings the shocks were delivered as programmed in E-prime.

Physiological measurements. Continuous measures of the physiological parameters were obtained using BIOPAC MP150, Biopac Systems, Goleta, CA, USA. Data was collected at a sampling rate of 500 Hz. The data was visually inspected and corrected for artifacts using AcqKnowledge 4.3.1 (Biopac Systems Inc.). A tailor-made toolbox in Matlab R2012b was used to extract the data as described below.

Skin conductance was recorded with two Biopac Systems Electrodes (EL507) filled with isotonic gel, attached to the medial phalanges of the ring and index finger of the left hand (185,271), which was not the side of shock delivery. A one-dimensional

median filter was applied to the raw signal. To obtain SCR magnitude, based on the phasic SCL, a SCR (in µS) was the maximum skin conductance level (SCL) in a seven s interval after stimulus onset, initiated in the first to fourth s minus the mean SCL during the first s after stimulus onset with a minimal change of 0.02 µS. SCRs below this threshold were considered to be zero (103,185). Then, a range correction was applied (184,185) using the maximum SCR from the US calibration phase (see below). Zero-responses were included in the analysis, hence we have used SCR magnitude.

The *electrocardiogram* was collected using two leads with Kendall Medi-Trace 200 Foam Electrodes (Covidien Ltd.) and a combfilter (50 Hz, Q = 5) was applied. After interpolation of the R spikes, a continuous signal for HR (in bpm) was obtained. For HR the average of seven s was used, which was the interstimulus interval, starting at stimulus onset per trial.

Blood pressure (in mmHg) was measured on the medial phalange of the middle finger of the left hand, using a finger cuff and collected with the Finometer MIDI (Finapres Medical Systems, Amsterdam, The Netherlands), which was connected to the BIOPAC MP150. A low-pass filter (2 Hz, Blackman 40 coefficients) was applied. For SBP and diastolic blood pressure (DBP) we used the averages of seven s starting at stimulus onset.

Baseline levels of tonic SCL, SBP, DBP, and HR were determined using the two last min of the five-min baseline measurement.

Design and procedure

The experimenter explained the procedure to the participants and attached them to the physiological equipment once they had provided informed consent. First, shock intensity levels were determined in the US calibration phase (for recommendations see 269). Participants were told that the shocks should be annoying up to a point where they were barely tolerable. The intensity was raised in small steps of 0.5 mA (or sometimes in steps of 0.1 mA for highly sensitive participants) in agreement with the participant to their maximum perceived level of annoyance or when the predetermined maximum of 15 mA had been reached. Shock intensity ranged from 0.8 to 15.0 mA (M = 5.29, SD = 2.71). Perceived US intensity was rated on a 10 point scale where ten indicated 'barely tolerable' (M = 8.4, SD = 0.83). The determined shock intensity was held constant throughout the experiment. Participants then filled out a demographical and biobehavioral questionnaire. This was followed by a habituation phase during which all images (including the mask) were presented twice in a fashion analogous to the subsequent acquisition trials; each trial started with a fixation cross in the middle of the screen for three s followed by the stimulus for 500 ms. After habituation participants rated the images for valence and arousal on a Visual Analogue Scale ranging from 0 to 100.

A five-min baseline period for the physiological measures followed during which a nature film was presented. Hereafter, two images were randomly selected to serve as CS+ and the other two served as CS-. To create contingency awareness, the participants were explicitly told which two images would be paired with a shock. This was followed by a repeated presentation of all images. This time for each image the participant had to indicate the extent to which they expected to receive a shock (269). The acquisition phase started once contingency awareness was confirmed by affirmative responses to the CS+ during this procedure. Two blocks of 16 trials each contained pseudorandom presentations of eight CS+ and eight CS-. The first trial was always a CS- and the second and last trial were always a reinforced CS+. The same image was never shown twice in a row (for recommendations see 269). The CS+ was partially reinforced in 75% of the trials to enhance resistance to extinction (272). For presentation of the US a delay conditioning procedure was used, providing a shock at 400, 440 or 480 ms of the 500 ms CS+ presentation. We used this specific fear conditioning paradigm to enhance differentiation and prevent extinction of the CR (e.g., 272-274).

The test phase of 12 trials immediately started after the last acquisition trial. It consisted of two blocks of six trials which consisted of solely CS+ or CS- images. The block order was counterbalanced. The first block was followed by a US-only trial with an interstimulus interval of 3 s to reinstate the conditioning effect. Participants were randomly assigned by E-prime to one of the presentation methods, to which the experimenters were blind. After the test phase participants had to rate the images again for valence and arousal.

After the test phase, we provided a forced choice prime recognition (AFC) task during which all four stimuli were presented as an awareness check. Participants were shown the four images five times in random order in a similar fashion as in the subliminal trials (i.e., 20 ms stimulus presentation and 500 ms mask presentation). To assess sensitivity, as objective measure of awareness, the proportion of correct responses was calculated (256). After each trial the participant had to indicate how well they could see the image on a scale from 1 ("I did not see the image at all") to 5 ("I could clearly see the image") and had to choose out of the four images the image they believed to have seen to address subjective awareness (121,122). Notably, this task did not assess contingency awareness, but awareness in terms of the perception of the stimuli. Finally, the participants were debriefed about the study goals and subliminal presentation of images. Before and after the experiment room temperature and humidity were noted and were found to be stable across participants.

Data reduction and statistical analyses

Differences in baseline biobehavioral characteristics of participants between presentation method were analysed with *t* tests, chi-square tests, and their

nonparametric equivalents, as appropriate. Changes in valence and arousal ratings were tested with independent *t* tests (post ratings minus pre ratings) and differences between stimulus types (CS+ versus CS-) was tested with paired *t* tests. A Bonferroni correction was applied, α = .0125. As a manipulation check, physiological differential responding (i.e., physiological responses to the unreinforced CS+ versus those to the CS-, 138,155,165) in the acquisition phase, was assessed by comparing the aggregated means of the responses to the CS types using a paired sample *t* test for all outcome measures, using the Benjamini-Hochberg correction for multiple comparison with the false discovery rate set at 10% (275-277). As main analyses, for the test phase multilevel analyses (MLA) were performed to assess the role of CS type (CS+ versus CS-) and presentation method (subliminal versus supraliminal) across the test trials. This particular method is useful as it enables analyzing data that change over time (e.g., 278). The change in physiological responding over the test trials (i.e., time) was modelled with CS type and presentation method as predictors. Significant changes in the Akaike information criterion (AIC) and Bayesian information criterion (BIC), based on chi-square tests, were used to determine the model fit (279). Analyses were performed with SPSS 23.0.

Results

Descriptive statistics

In several cases technological difficulties prevented adequate measurement of one or more physiological outcome measure. The assumptions for the analyses were checked. Outliers for the physiological parameters (> 3 *SD*s) were coded as missing values for the respective variables based on the data points of all trials and participants, following the hierarchical structure of the data for the MLA (e.g., 278). Furthermore, three participants displayed BP values that were considered to be extreme (SBP > 175 and/or DBP > 110) and the relating blood pressure variables were not included in the analysis. The data were considered to be missing at random. Participants reported not to have seen the subliminal stimuli ($M = 1.26$, $SD = 0.343$) in the awareness check, suggesting that the subliminal presentation of the stimuli had been successful (122). However, the results from the AFC indicated that one participant correctly identified 75% of the images in the awareness check, despite not reporting to have seen the images (256). Analyses were performed with and without this participant, which did not result in meaningful differences, and those including this participant are reported. Furthermore, the sample consisted mostly of Western Europeans ($n = 72$, 77.4%). Age was slightly higher in the subliminal group ($M = 21.2$, $SD = 2.50$) compared with the supraliminal group ($M = 20.0$, $SD = 2.81$, Mann-Whitney $U = 723$, $Z = 2.68$, $p = .007$, $r = .278$). No other differences between groups were found. The final number of cases for each outcome measure and other baseline characteristics are presented in Table 1.

TABLE 1 Baseline characteristics stratified by presentation method

	Total			Subliminal			Supraliminal				
Measure	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*	*n*	t/χ^2	*r*
Demographics											
Age, years[a]	20.6	2.73	93	21.2	2.50	41	20.0	2.81	52	2.68**	.278
Female sex[b]	68	(73)	93	29	(71)	41	39	(75)	52	-0.21	.048
BMI	22.2	2.92	93	22.1	2.11	41	22.2	3.44	52	-0.11	.011
Biobehavioral variables											
Smoking[b]	19	(20)	93	7	(17)	41	12	(23)	52	-0.51	.074
Drugs[b]	13	(14)	93	7	(17)	41	6	(12)	52	0.58	.079
Cafeine use (average/day)	1.57	0.87	65	1.63	0.95	30	1.51	0.78	35	0.55	.069
Alcohol use (average/week)	3.54	3.72	93	3.66	4.71	41	3.44	2.74	52	0.28	.029
General practitioner (visits last 6 months)	1.22	1.59	93	1.37	1.77	41	1.10	1.43	52	0.81	.084
Cardiovascular measures											
Tonic SCL[c]	2.20	0.64	92	2.21	0.70	41	2.18	0.55	51	0.20	.021
SBP	127.8	16.7	87	127.1	17.4	38	128.2	16.3	49	-0.30	.032
DBP	70.1	8.85	88	69.7	9.12	39	70.4	8.71	49	-0.38	.041
HR	75.0	10.2	92	73.8	10.6	40	76.0	9.92	52	-1.02	.106
Personality[d]											
Trait anxiety	39.9	8.09	85	40.4	8.40	35	39.6	7.94	50	0.46	.050
Trait worry	48.9	12.3	85	51.1	12.7	35	47.3	11.9	50	1.43	.154

Note. The cell sizes are displayed since the amount of usable recordings varied across outcome measures. All tests were performed two-sided. Age was higher in the subliminal group. *Abbreviations*: BMI = Body mass index, GP = General practitioner, SCL = Skin conductance level, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate.

[a] Mann-Whitney U test was performed as nonparametric test, the *Z* statistic and *r* as effect size were provided.

[b] Displayed are the number of positive responses (with percentage between brackets), Pearson χ^2 was used as test statistic and phi as effect size.

[c] Square root transformation was applied. Note that this baseline assessment represent a different aspect of the skin conductance activity than skin conductance magnitude and as a consequence has different properties (see 185).

[d] Trait anxiety was measured with the State-Trait Anxiety Inventory, Trait version (STAI-T; 280) and trait worry with the Penn State Worry Questionnaire (208).

** $p < .01$

Valence and arousal ratings of the stimuli

Prior to the fear conditioning the stimuli were rated low in arousal (Book: *M* = 17.9, *SD* = 20.0; Pegs: *M* = 13.6, *SD* = 14.7; Car: *M* = 28.4, *SD* = 22.0; Spoon: *M* = 16.3, *SD* = 17.3; Mask: *M* = 32.1, *SD* = 25.5) and neutral in valence (Book: *M* = 56.5, *SD* = 21.7; Pegs: *M* = 49.2, *SD* = 14.1; Car: *M* = 53.6, *SD* = 18.6; Spoon: *M* = 58.3, *SD* = 16.6; Mask: *M* = 55.7, *SD* = 17.5).

After the fear conditioning, participants rated the CS+ images as more arousing (*M* change = 27.0, *SD* = 25.3, $t(67) = 8.76$, $p < .001$, $r = .731$) and more negative (*M* change = -17.9, *SD* = 20.5, $t(67) = -7.15$, $p < .001$, $r = .658$). The CS- images were rated low in arousal (*M* change = 1.51, *SD* = 13.5, $t(67) = 0.921$, $p = .360$, $r = .112$), which was comparable to the preconditioning ratings, and slightly, but not statistically significant considering α = .0125, more positive (*M* change = 4.01, *SD* = 15.2, $t(67) = 2.17$, $p = .034$, $r = .256$). The CS+ were, compared with the CS-, rated as more arousing (*M* difference = 25.5, *SD* = 30.3, $t(66) = 6.90$, $p < .001$, $r = .647$) and more negative (*M* difference = -21.9, *SD* = 29.4, $t(66) = -6.09$, $p < .001$, $r = .600$). Finally, differences in changes in ratings of arousal and valence between subliminal and supraliminal presentation were small and statistically nonsignificant (*r*s < .20, *p*s > .10).

Manipulation check

The CS+ elicited a higher mean SCR magnitude (*M* = 0.189, *SD* = 0.237) compared with the CS- (*M* = 0.063, *SD* = 0.086; $t(92) = 6.08$, $p < .001$, $r = .535$, a log transformation was applied). See Figure 1. The CS+ did not elicit a higher mean SBP level (*M* = 132.9, *SD* = 16.4) compared with the CS- (*M* = 133.2, *SD* = 15.9, $t(85) = -0.762$, $p = .224$, $r = .082$), nor a higher mean DBP level (CS+: *M* = 72.2, *SD* = 9.00; CS-: *M* = 72.2, *SD* = 9.03, $t(87) = 0.016$, $p = .494$, $r = .002$). However, the CS+ did elicit a small, statistically marginally significant, decrease in mean HR level (*M* = 75.7, *SD* = 9.41) compared with the CS- (*M* = 76.2, *SD* = 9.91, $t(89) = -1.98$, $p = .050$, $r = .205$), which was opposite of what was expected. See also Table 2.

Test phase

Multilevel modeling was applied to the outcome measures in the test phase. In all the models the values per trial and related baseline measure were grand mean centered and an autoregressive covariance structure was applied to the error variance, as is appropriate for fitting growth models (see for example 278). Age and Order (of the blocks) was examined as predictor in the models of all the outcome measures but did not increase model fit and results are reported without Age and Order. Since the residuals of the final models were normally distributed, in contrast to the acquisition phase, no transformations had to be applied before the model fitting procedure (278). A basic growth model was fitted to the data to model the change of time, that is, across trials (Model 1; see for example 278), which served as the basic model to

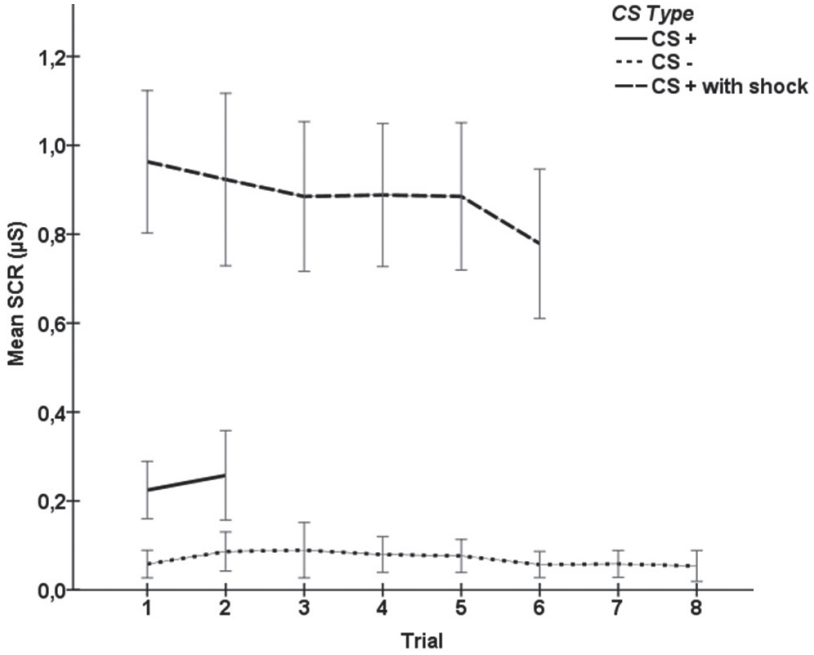

FIGURE 1 SCR magnitude (µS) in the acquisition phase for all CS types. Trial is presented as corresponding trial for each CS type and not in the actual order in which they were displayed in the experiment since stimuli were presented pseudo-randomly. The CS+ with US trials indicate the mean SCR magnitude when the CS+ was combined with the US (i.e., a shock) and display the unconditional response. The CS+ trials indicate the response to the CS+ in absence of the US and display the conditional response. Errors bars display the 95% Confidence Interval. A difference across trials between the CS+ and CS- can be observed ($t(92) = 6.08$, $p < .001$, $r = .535$). *Abbreviations*: SCR = Skin conductance response, µS = microsiemens, CS = Conditional stimulus, US = Unconditional stimulus

TABLE 2 Paired sample *t* tests of the physiological outcome measures during the acquisition phase

	CS+		CS-				
	M	*SD*	*M*	*SD*	*N*	*t*	*r*
SCR magnitude[a]	0.189	0.237	0.063	0.086	93	6.08***	.535
SBP	132.9	16.4	133.2	15.9	86	-0.762	.082
DBP	72.2	9.00	72.2	9.03	88	.016	.002
HR	75.7	9.41	76.2	9.91	90	-1.98	.205

Note. SCR magnitude was larger in response to the CS+ compared with the CS-. To correct for multiple comparisons the Benjamini-Hochberg correction was used with the false discovery rate set at 10% (275-277). *Abbreviations*: SCR = Skin conductance response, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, CS = Conditional stimulus.

[a] The data was log transformed.

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

which the others were compared. To test the hypotheses, first CS type (CS+ or CS-) was added to the model (Model 2) as well as its interaction with trial number (CS type×Trial; Model 3). Then Presentation method (i.e., subliminal and supraliminal) was added (Model 4) and its interactions with trial number (Presentation×Trial; Model 5). Finally, we checked for a CS type×Presentation interaction (Model 6).

For SCR magnitude, the model with a linear trend (Trial) showed the best fit to the data (Model 1). Although Trial was not significant in the model, a quadratic trend did not improve the model fit. Figure 2A displays the course of the mean SCR magnitude across trials in the test phase for CS type and Presentation method. Model 2 showed the best model fit ($\Delta AIC = 7.9$, $p < .01$ and $\Delta BIC = 3.1$, $p < .10$ compared to Model 1). Furthermore, a statistical significant negative association of SCR magnitude was found with CS type ($B = -0.061$, $t(333.5) = -3.19$, $p = .002$). The results are displayed in Table 3. Notably, this finding was confirmed by a post hoc MLA in the subliminal group only. Again, Model 2 was the best fit, $\Delta AIC = 7.3$, $p < .01$ and $\Delta BIC = 3.5$, $p < .10$ compared to Model 1, and CS type was statistically significantly association with SCR magnitude ($B = -0.089$, $t(208.2) = -3.09$, $p = .002$). This indicates that during the test phase, the CS+ elicited a higher SCR magnitude compared with CS-, both supraliminally and subliminally.

Even though we did not find differential BP responses in the acquisition phase, we performed the multilevel analyses on the test phase since they addressed our main hypothesis. For SBP the slope across trials was allowed to vary randomly between participants. Figure 2B displays the course of the mean SBP level across trials for CS type and Presentation. The results are displayed in Table 4. Statistical significant associations with SBP were found for Trial and Trial^2 which indicates a linear decrease and quadratic change (Model 1). None of the models showed a better fit to the data, but when fitting Model 4 a statistically significant effect of Presentation on SBP was apparent ($B = 3.04$, $t(74.7) = 2.05$, $p = .044$). This may indicate that during the test phase CS+ and CS- elicited equal SBP changes that were higher when the CSs were presented subliminally.

For DBP the slope across trials was allowed to vary randomly between participants. Figure 2D displays the course of the mean DBP level across trials for CS type and Presentation. The results are displayed in Table 5. Statistical significant associations with DBP were found for Trial and Trial^2 which indicates a linear increase and quadratic change (Model 1). Model 2 improved the model fit, $\Delta AIC = 7.8$, $p < .01$ and $\Delta BIC = 3.1$, $p < .10$ compared to Model 1, with an association of CS type with DBP in the opposite direction of what was expected ($B = 0.54$, $t(245.3) = 3.17$, $p = .002$). This indicates that during the test phase CS+ did not elicit the expected increase in DBP, irrespective of group, but a DBP decrease. Although Figure 2D suggests that this decrease occurred during earlier trials for the subliminal presentations, the models with Presentation×Trial did not improve the fit to the data.

For HR the slope across trials was allowed to vary randomly between participants. Figure 2C displays the course of the mean HR level across trials for CS type and Presentation. The results are displayed in Table 6. A statistical significant association was found for Trial which indicates a linear increase (Model 1). Adding CS type to the model (Model 2) improved the model fit, $\Delta AIC = 4.4$, $p < .05$ and $\Delta BIC = -0.5$, $p > .25$ compared to Model 1, with a significant association of CS type with HR in the opposite direction ($B = 0.67$, $t(267.6) = 2.55$, $p = .011$). This indicates that presentations of the CS+, compared to CS-, did not lead to increased HR levels, as expected, but to decreased HR levels.

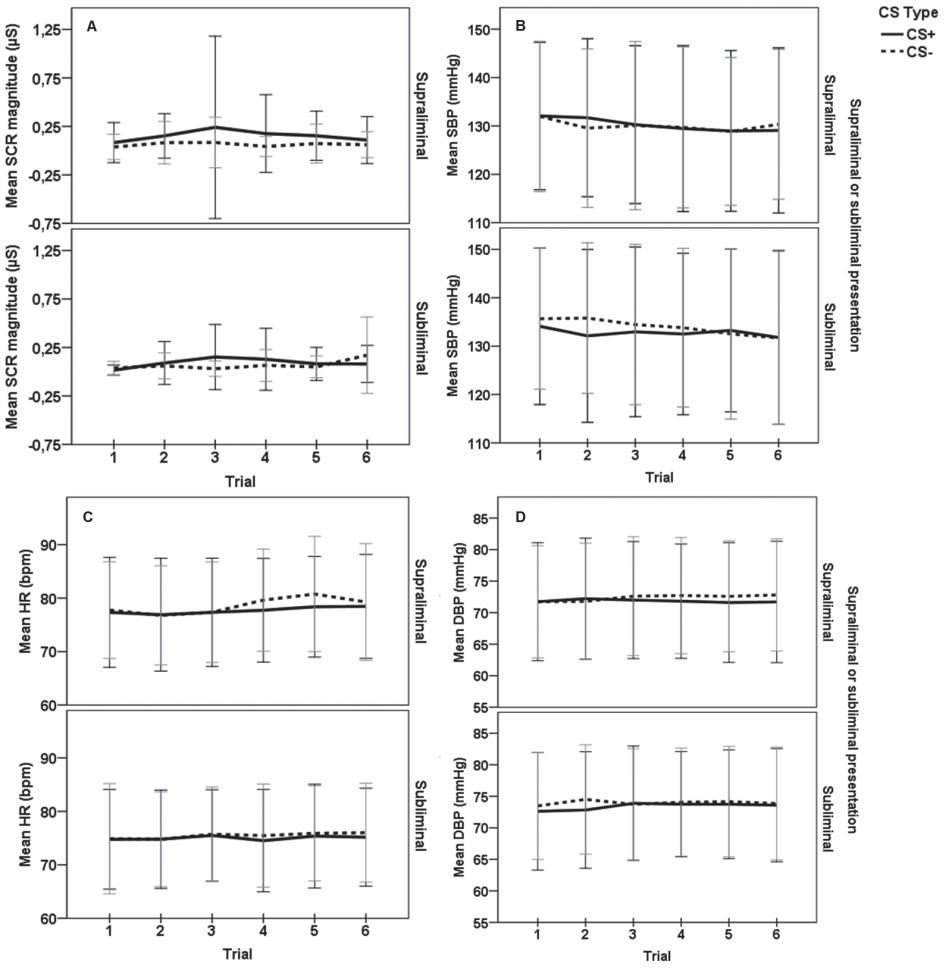

FIGURE 2 Test phase displayed for the CS+ and CS- presentation method for all outcome measures (A. SCR magnitude (µS), B. SBP (mmHg), C. HR (bpm), D. DBP (mmHg). The CS types were presented in two adjacent blocks of six trials, either subliminally or supraliminally, which are aggregated per trial number within the blocks. Error bars are +/- 1 SD. *Abbreviations*: CS = Conditional stimulus, SCR = Skin conductance response, µS = Microsiemens, SBP = Systolic blood pressure, HR = Heart rate, DBP = Diastolic blood pressure

TABLE 3 Summary of multilevel analysis of SCR magnitude (μS) during the test phase

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	0.084	0.019	4.46***	0.17	0.034	5.10***	0.19	0.054	3.49***	0.23	0.067	3.47***	0.27	0.076	3.57***	0.41	0.112	3.68***
Trial	0.006	0.005	1.09	0.006	0.005	1.15	0.0005	0.017	0.028	0.0005	0.017	0.028	-0.016	0.023	-0.69	-0.016	0.023	-0.68
Baseline SCL	0.055	0.021	2.61*	0.056	0.021	2.62*	0.056	0.016	2.62*	0.056	0.021	2.67**	0.056	0.021	2.67**	0.056	0.021	2.67**
CS type				-0.061	0.019	-3.19*	-0.071	0.034	-2.08+	-0.071	0.034	-2.09*	-0.071	0.034	-2.09*	-0.16	0.064	-2.55*
CS type × Trial							0.004	0.011	0.33	0.004	0.011	0.33	0.004	0.011	0.34	0.004	0.011	0.34
Presentation										-0.031	0.028	-1.11	-0.060	0.038	-1.56	-0.16	0.070	-2.28**
Presentation × Trial													0.012	0.011	1.10	0.011	0.011	1.08
CS type × Presentation																0.066	0.039	1.70+
AIC		407.4			399.5			401.4			402.2			402.9			402.1	
BIC		436.7			433.6			440.4			446.0			451.7			455.7	
N		6			7			8			9			10			11	

Note. Error at Level-1 was organized with a first-order autoregressive covariance structure. At Level-2 the covariance was unstructured structure was specified. Trial was centered and Baseline SCL was grand mean centered. The model included a random intercept. The SCR residuals were normally distributed. Baseline SCL was square root transformed. CS type was either CS+ or CS-. Presentation was either subliminal or supraliminal. The Models were compared using the chi-square statistic with $df = 1$. Model 2 was considered to have the best fit with $\Delta AIC = 7.9$, $p < .01$, $\Delta BIC = 3.1$, $p < .10$. *Abbreviations*: SCR = Skin conductance response, SCL = Skin conductance level, μS = Microsiemens, CS = Conditional stimulus, AIC = Akaike information criterion, BIC = Bayesian information criterion, *N* = Number of parameters.

$^{+}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

TABLE 4 Summary of multilevel analysis of SBP (mmHg) during the test phase

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	132.7	0.77	171.8***	132.5	0.94	141.3***	132.5	1.19	111.0***	128.3	2.40	53.4***	128.4	2.41	53.2***	130.2	2.85	45.7***
Trial	-1.02	0.26	-3.90***	-1.03	0.26	-3.90***	-1.06	0.38	-2.79***	-1.06	0.38	-2.79*	-1.13	0.52	-2.17*	-1.12	0.52	-2.14*
Trial × Trial	0.10	0.046	2.23*	0.10	0.046	2.23*	0.10	0.046	2.21*	0.10	0.046	2.21*	0.10	0.046	2.21*	0.10	0.047	2.21*
Baseline SBP	0.86	0.046	18.9***	0.86	0.046	18.9***	0.86	0.046	18.9***	0.86	0.045	19.4***	0.86	0.045	19.4***	0.86	0.045	19.4***
CS type				0.17	0.36	0.46	0.10	0.61	0.17	0.099	0.61	0.16	0.10	0.61	0.16	-1.16	1.19	0.97
CS type × Trial							0.026	0.20	0.13	0.026	0.20	0.13	0.026	0.20	0.13	0.026	0.20	0.13
Presentation										3.04	1.48	2.05*	3.01	1.49	2.01*	1.68	1.84	0.91
Presentation × Trial													0.050	0.26	0.20	0.041	0.26	0.16
CS type × Presentation																0.90	0.73	1.23
AIC	5421.1			5422.9			5424.9			5422.8			5424.8			5425.3		
BIC	5459.5			5466.1			5472.8			5475.5			5482.3			5487.6		
N	8			9			10			11			12			13		

Note. Error at Level-1 was organized with a first-order autoregressive covariance structure. At Level-2 a variance components structure was specified. Trial was centered and Baseline SBP was grand mean centered. The model included a random intercept and slope. CS type was either CS+ or CS-. Presentation was either subliminal or supraliminal. The models did not provide a better fit compared with Model 1. *Abbreviations*: SBP = Systolic blood pressure, CS = Conditional stimulus, AIC = Akaike information criterion, BIC = Bayesian information criterion.

$^{+}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

TABLE 5 Summary of multilevel analysis of DBP (mmHg) during the test phase

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	71.6	0.38	186.1***	70.8	0.46	153.5***	71.1	0.59	121.1***	70.1	1.20	58.6***	70.1	1.21	58.1***	70.3	1.41	49.8***
Trial	0.397	0.15	2.71***	0.39	0.15	2.68**	0.26	0.20	1.30	0.26	0.20	1.30	0.20	0.26	0.74	0.20	0.26	0.74
Trial × Trial	-0.06	0.03	-2.24*	-0.060	0.027	-2.24*	-0.062	0.027	-2.29*	-0.062	0.027	-2.29*	-0.062	0.027	-2.29*	-0.062	0.027	-2.29*
Baseline DBP	0.974	0.04	22.3***	0.97	0.044	22.4***	0.97	0.044	22.2***	0.97	0.043	22.3***	0.97	0.043	22.3***	0.97	0.043	22.3***
CS type				0.54	0.17	3.17**	0.31	0.30	1.04	0.31	0.30	1.04	0.31	0.30	1.04	0.21	0.57	0.36
CS type × Trial							0.093	0.097	0.96	0.094	0.097	0.96	0.093	0.097	0.96	0.093	0.097	0.96
Presentation										0.77	0.74	1.04	0.72	0.75	0.96	0.61	0.92	0.67
Presentation × Trial													0.047	0.12	0.39	0.047	0.12	0.38
CS type × Presentation																0.07	0.35	0.21
AIC		4355.0			4347.2			4348.2			4349.2			4351.0			4353.0	
BIC		4393.5			4390.4			4396.3			4402.0			4408.7			4415.5	
N		8			9			10			10			12			13	

Note. Error at Level-1 was organized with a first-order autoregressive covariance structure. At Level-2 a variance components structure was specified. Trial was centered and Baseline DBP was grand mean centered. The model included a random intercept and slope. CS type was either CS+ or CS-. Presentation was either subliminal or supraliminal. Model 2 was the best fit with $\Delta AIC = 7.8$, $p < .01$ and $\Delta BIC = 3.1$, $p < .10$ compared to Model 1. *Abbreviations*: DBP = Diastolic blood pressure, CS = Conditional stimulus, AIC = Akaike information criterion, BIC = Bayesian information criterion, *N* = Number of parameters.

$^{+}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

TABLE 6 Summary of multilevel analysis of HR (bpm) during the test phase

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	75.2	0.35	214.8***	74.2	0.52	141.4***	75.1	0.77	97.9***	76.7	1.21	63.3***	76.0	1.25	60.6***	75.1	1.67	44.8***
Trial	0.36	0.076	4.74***	0.35	0.076	4.66***	-0.023	0.24	-0.10	-0.02	0.23	-0.10	0.42	0.31	1.35	0.42	0.31	1.33
Baseline HR	0.90	0.036	25.4***	0.90	0.036	25.4***	0.90	0.036	25.4***	0.90	0.035	25.6***	0.90	0.034	25.7***	0.90	0.034	25.7***
CS type				0.67	0.26	2.55*	0.048	0.46	0.11	0.048	0.45	0.11	0.048	0.46	0.11	0.68	0.88	0.78
CS type × Trial							0.25	0.15	1.69+	0.25	0.15	1.69+	0.25	0.15	1.69+	0.25	0.15	1.69+
Presentation										-1.08	0.67	-1.62	-0.60	0.70	-0.85	0.065	1.06	0.062
Presentation × Trial													-0.32	0.15	-2.15*	-0.31	0.15	-2.12*
CS type × Presentation																-0.45	0.53	-0.84
AIC		5435.5			5431.1			5430.3			5429.7			5427.2			5428.5	
BIC		5469.6			5470.1			5483.2			5488.5			5491.9			5487.0	
N		7			8			9			10			11			12	

Note. Error at Level-1 was organized with a first-order autoregressive covariance structure. At Level-2 a variance components structure was specified. Trial was centered and Baseline HR was grand mean centered. The model included a random intercept and slope. CS type was either CS+ or CS-. Presentation was either subliminal or supraliminal. Model 2 provided the best fit with $\Delta AIC = 4.4$, $p < .05$ and $\Delta BIC = -0.5$, $p > .25$ compared to Model 1. *Abbreviations*: HR = Heart rate, CS = Conditional stimulus, AIC = Akaike information criterion, BIC = Bayesian information criterion, *N* = Number of parameters.

$^{+}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Discussion

To test whether stress-related cognition outside of awareness, here referred to as unconscious stress, increases physiological responses, we presented fear conditioned images (CS+) below the threshold of awareness (subliminally). The manipulation check indicated that fear conditioning was successful, as SCR magnitude was larger in response to the CS+ (stress-related) compared with the CS- (stress-unrelated) images. However, differences in BP and HR were small in response to both CS types. During the test phase, the response to both the subliminal and supraliminal CS+ was again greater for SCR magnitude, but not for the CV variables. Moreover, the DBP and HR were smaller in response to the CS+ rather than larger. This is the first study to examine the effect of unconscious stress on health-relevant outcome measures using a fear conditioning paradigm. The findings indicate that the representation of a stressor that result from fear conditioning can increase electrodermal responding even when the stressor was presented subliminally. Although the effect was not convincingly found for CV activity, this study partly confirms that unconscious stress may affect the physiological state. The increases in SCR magnitude after subliminal CS+ presentation are in line with previous research (for a review see 203). Importantly, though, most of these studies used fear-relevant stimuli as the CS+, such as images of guns, while we successfully induced increases in SCR magnitude using fear-irrelevant, or neutral, stimuli. We replicated the findings of two early studies that used less convincing subliminal presentations, as argued in the introduction (111,128). In other words, different SCR magnitudes were observed in response to the CS+ versus CS- stimuli, throughout the test phase, even with the use of fear-irrelevant stimuli. This finding disputes the 'preparedness' theory that states that only evolutionary relevant stimuli would result in CRs that are resistant to extinction (e.g., 267,268). Other factors than their intrinsic fear relevance, such as the intensity of the US, the CS-US interval, timing of the UCS presentation relative to the CS (i.e., delay or trace conditioning), and controllability of the US (267) may explain this prolonged differential responding to the CS+ versus CS- stimuli, even during subliminal presentations. Furthermore, the findings are in accordance with the conventional interpretation of increased SCR as an orienting response to novel or significant stimuli (103). This would be consistent with the finding of a lower HR in response to relevant items (282), as will be discussed below. Thus, the differential effect between CS+ and CS- on SCR magnitude indicates that the conditioning procedure effectively enhanced the significance of the stimuli.

Against our expectations, only small effects were found on BP and HR during the acquisition. One probable explanation is that the CS+ was not sufficiently stressful. Perhaps this was due to the intensity of the US, the shock. Although participants were expected to indicate when they could barely tolerate the intensity of the shock, they were inclined to set the intensity of the US lower than what they would be able to handle as can be concluded from the exit questionnaire that the participants filled

out. Still, a differentiated response between CS types was apparent on SCR magnitude and changes in ratings of valence and arousal. Although it is not likely that the suboptimal intensity affected the findings, the aversiveness of the US should have been rated after fear acquisition and the test phase. In general, as also suggested by Lonsdorf et al. (2017, 269), standardization of the methods for US intensity calibration is called for and progress in this area should be monitored and implemented to benefit future studies. Furthermore, it is also likely that the 'preparedness' theory (e.g., 267,268) mentioned above may hold for slower and less sensitive physiological variables than electrodermal responses. Finally, in this study we have used BP and HR as CV outcome measures, but previous studies have indicated that other physiological parameters respond to stressors as well (202). Moreover, Van der Ploeg et al. (2017, 248) found an effect of subliminal threatening versus neutral words on total peripheral resistance, which has been related to adverse health outcomes (97,98). Perhaps it is more sensitive to subtle threat cues and future studies on unconscious stress should consider including total peripheral resistance as outcome measure. Thus, the fear-irrelevant, or neutral, stimuli in combination with an insufficiently intense US, may have contributed to the restriction of the fear conditioning effects to SCR magnitude.

Several unexpected findings require some elaboration. Despite an absence of differentiation between the CS types, SBP was generally higher when the stimuli were presented subliminally than when presented supraliminally. To our knowledge no other studies have been performed using fear conditioning and subliminal presentation while measuring SBP that can help explain this finding. To the participants with subliminal presentation of the CSs, the testing phase consisted of a sequence of 'masks' and one US. This may have led to a state of uncertainty and vigilance resulting in a higher SBP. Another possibility is that the participants put in more effort in the subliminal group to clearly see what was presented. Furthermore, in contrast to our expectations, a *lower* DBP level in response to the CS+ was apparent in the supraliminal and subliminal condition, in the testing phase only. In general, DBP increases in response to stressors (281). However, previous studies provide less consistent results regarding changes in BP when people are viewing arousing pictures (102) and decreases in BP to negative affective pictures have also been observed (102, Figure 1). These inconsistencies call for more research on the effect of appetitive and aversive stimuli on BP.

Finally, also against our expectations, in response to the CS+ HR was *lower* during the acquisition phase and in the test phase. Furthermore, DBP was *lower* rather than higher in response to the CS+ during the test phase. Since this is the first study, to our knowledge, measuring DBP continuously during a fear conditioning procedure, we can offer no explanation. The findings regarding HR most likely represent an orienting response (282-284). More specifically, it has been suggested that different characteristics of the HR response reflect different effects of conditioning procedures (285), including immediate HR deceleration (due to the orientation reflex) followed

by HR acceleration. Furthermore, in a series of fear conditioning studies Castegnetti et al. (2016, 286) used HR period as index of fear memory in addition to SCR and found that overall the CS+ compared to the CS- elicited a small decrease at trial onset and a steep acceleration after 4.7 s following trial onset. Moreover, an enhanced acceleration may represent the physiological mobilization to avoid a threatening situation (285,287,288). Enhanced HR acceleration (and a relative absence of an initial HR decrease) has been observed in PTSD patients when presented with negative affective pictures (289). The current findings with HR here may have been due to an overrepresentation of the initial deceleration and may reflect an adaptive orienting reaction to the presentation of salient information. Then, a conclusion would be that the stimuli were not stressful enough to evoke the typical defensive fight/flight response. Thus, while the CS+ appears to have been perceived as sufficiently relevant to lead to an enhanced orienting response, it might not have been sufficiently stressful to increase physiological responding beyond initial HR deceleration and SCR magnitude increases and the physiological effects of fear conditioning may be limited to reflexive processes rather than sustained adverse physiological activation.

The findings should be interpreted with several limitations in mind. First, in the test phase the US was again presented, without combining it with an image, to reinstate the CR. The US was presented between the two blocks, which could have led to anomalies on the first trial. Regarding SCR magnitude for example, due to the shock SCL may have been high already and precluded effects on the first trial. In general, this may have affected the effects across trials, but would not affect the differentiation between the CS+ and CS- since order of presentation blocks was randomized. Although in depth analyses on this dataset did not indicate an order effect, future studies should execute the interstimulus interval carefully when using a reinstatement protocol. Second, the acquisition phase consisted of 32 trials, which can be considered as long and may lead to habituation within the acquisition phase and a diminished response in the test phase (269). However, considering the results regarding SCR magnitude we believe to have sufficiently maintained the CR, which is probably due to the pseudo-random presentation procedure. Third, as Lovibond and Shanks (2002, 195) have argued, the subliminal presentation of stimuli does not necessarily prohibit the participant from distinguishing the CS+ and CS- at some level of processing. This may lead to mistakenly ascribe effects to the subliminal nature of the trials. More elaborate awareness checks for example based on feature detection (e.g., pairing subliminal and supraliminal stimuli) and/or standardized confidence ratings could lead to advanced conclusions on unconscious processes (e.g., 122). However, the issue raised by Lovibond and Shanks (2002, 195) is based on work by Öhman and colleagues (e.g., 153). The current work is different: the stimuli were tested in a separate pilot study on features detection during subliminal presentation, the two CS+s were random combinations of four neutral images, the acquisition phase took

place supraliminally, and the reinstatement trial was implemented. Thus, it seems unlikely that the participants could discriminate the CSs by other means during the subliminal presentation. Finally, by employing a habituation phase, we may have unintentionally evoked latent inhibition, that is, impeded acquisition of the CR due to pre-exposure to the to-be conditional stimuli (290). This might have led to less pronounced effects, even though the conditioning procedure was effective. Although we intentionally included the habituation trials to prevent orientation responses to the stimuli during acquisition, this strategy may be reconsidered in future studies (i.e., by presenting the images once instead of twice, see also 269), especially since it appears that an orientation response still occurred.

Notably, this study is unique in the field of stress and health by using fear conditioning to induce stress and measure health-relevant outcomes. This provides a study design that allows the researcher to create a stressor that can be considered equal across participants but is tailored to the participant. In for example the study by Van der Ploeg et al. (2016, 202) participants performed a counting task and received angry feedback to induce stress and in the study by Van der Ploeg et al. (2017, 248) participants viewed validated threat and neutral words. Although these and other methods (see for an overview 92) have been widely used to induce a stress response, they assume that all participants show a similar stress response to these stressors. However, the associations with the used stressor may greatly differ across participants. Moreover, individual sensitivity to these stressors is hard to quantify. Here, in contrast, the association was created in the laboratory, was the same across participants, and the sensitivity to the created stressor could be qualified and taken into account (e.g., by dealing with nonresponders). However, it must be noted that fear conditioning can be challenging to achieve and researchers are faced with a lack of standardization and consensus within the field (Van der Ploeg et al., 2017, 203). Moreover, the limitations discussed above should be adequately dealt with as suggested. The interested reader is referred to the comprehensive work of Lonsdorf et al. (2017, 269) for methodological considerations. In sum, fear conditioning provides a new and promising method to study the effect of psychological stress on physiology.

To conclude, this is the first study to address unconscious stress and the effect on health-relevant parameters using a fear conditioning paradigm. By pairing neutral images with a shock and presenting these conditional images subliminally, we expected to find larger physiological responses to the newly created stressor. Although the SCR magnitude was larger in response to the subliminally presented stress-related images (CS+) compared to the stress-unrelated images (CS-), the findings for BP and HR were not that straightforward. In sum, unconscious stress, here operationalized as subliminally presented fear conditioned stimuli, can affect the physiological state but at the same time may not, based on the current study design, instigate health-relevant changes.