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

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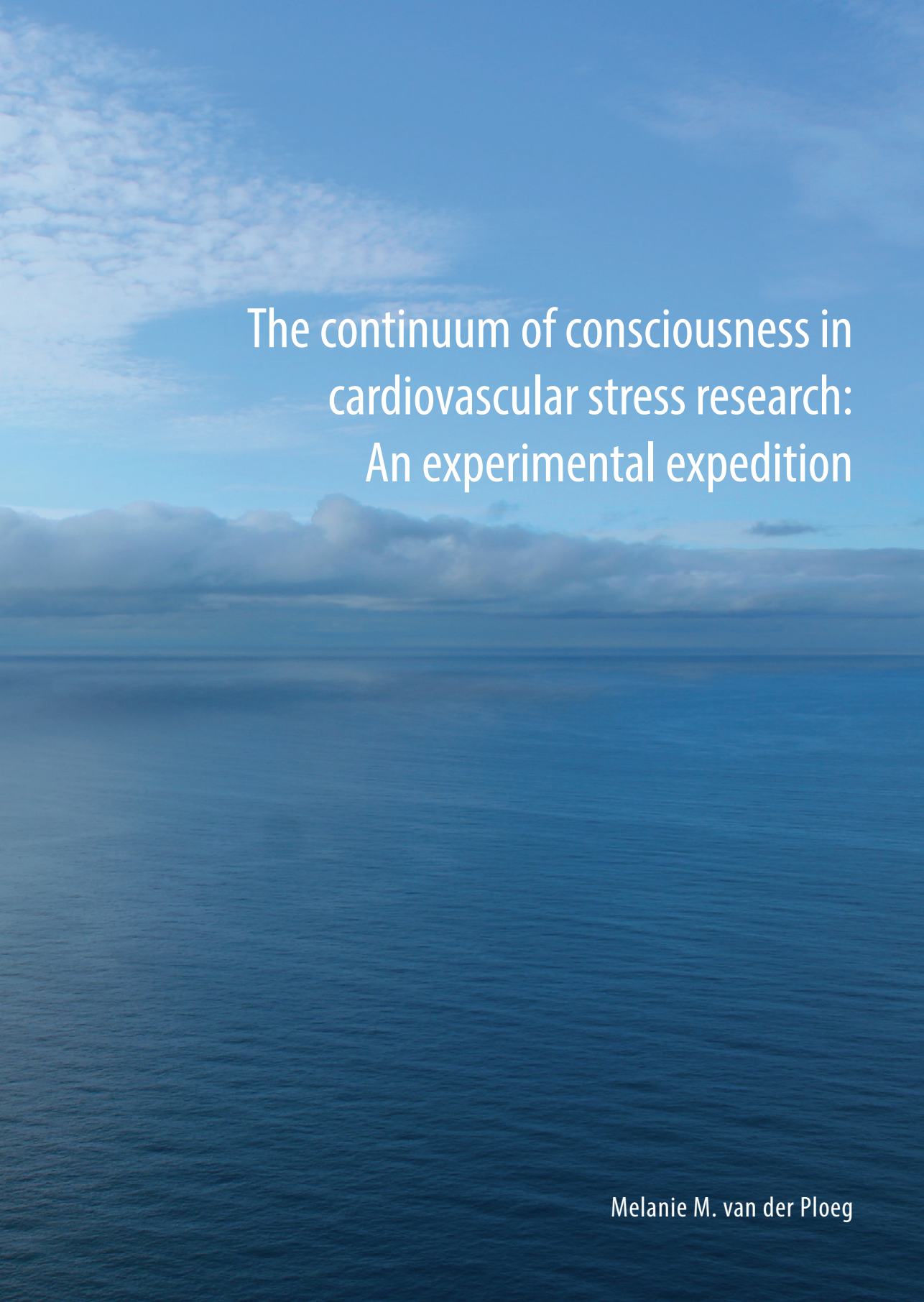

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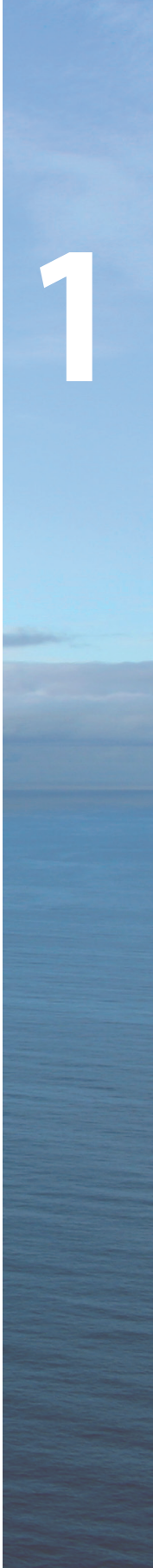
Content

Chapter 1

Introduction



Cardiovascular (CV) diseases are prevalent and place a major burden on individuals and health care resources. Treatment is usually aimed at traditional risk factors such as diabetes mellitus, smoking, and physical inactivity (1). Psychological stress has been found to negatively affect health and contributes to the development and worsening of somatic diseases, such as CV diseases (e.g., 2-7), but the underlying mechanisms are still unclear (e.g., 8,9). It is widely recognized that these adverse consequences are largely the result of chronic stressors and impaired physiological recovery (10-16). One of the mechanisms underlying this relationship is thought to be the ongoing cognitive representations of the stressor, which is referred to as perseverative cognition. Perseverative cognition can manifest itself for example as worrying (17-21). However, these perseverative cognitions do not fully explain the effect of psychological stress on health-relevant outcomes (e.g., 22-25). It has been suggested that stress-related cognitions can also occur outside of awareness, which is referred to as unconscious stress (26-28). In the current thesis, the effect of stress-related cognitions occurring outside of awareness on physiological activity is explored with a systematic review and a series of experimental studies to enhance our understanding of the relationship between psychological stress and health.

In this Chapter, I will elaborate on the three main components of this thesis; stress, awareness, and health-relevant parameters. This is followed by a discussion of the main methodological issues that play a key role in this area of research. Finally, I will introduce the content of the remainder of this thesis in an outline.

The concept of stress

Research on psychological stress has been hindered by a lack of consensus on the definition of *stress* (e.g., 29,30). Consequently, a wide range of operationalizations of psychological stress is used, such as perceived stress, worry, anxiety, or anger (e.g., 17,20,31-33). According to Levine and Ursin (1991, 34), these operationalization are all valid methods to test specific stress-related hypotheses, but an overarching formal definition is called for. The idea of a formal and systematic definition was further developed into the cognitive activation theory of stress (CATS; 16), which defines *stress* based on four components: stress stimuli (or stressors), stress experience (i.e., appraisal), the (nonspecific) stress response, and the feedback from the stress response which generates stress-related behavior and cognition. In CATS, the stress response is an adaptive process that induces increased arousal and concurrent behavioral and physiological changes. This occurs when the organism detects a situation that does not meet its expectancies, which constitutes the stressor. When the organism is unable to cope with the situation the response is maladaptive, which leads to continuous arousal, and may lead to negative cognitive states such as helplessness, hopelessness, and negative affect resulting from the continuous negative physiological feedback. Notably, the CATS emphasizes the general and nonspecific nature of the

physiological and affective response to stress stimuli (16). If one reads this definition closely, it becomes apparent that the psychological aspects encompasses often segregated concepts such as cognition or emotion, as the CATS assumes that all of these responses are part of the (mal)adaptive processes. Within this framework, any stimulus that induces negative affect is seen as a stressor. So, stress is considered to be a psycho-biological response set to a stressor. In the current thesis, the psychological aspects of the response as described by the CATS are further explored to explain its relationship with somatic disease.

The stress response is quickly activated in response to a stressor, but when one further appraises this stressor as something that should be dealt with effectively, the activation of the stress response is short-lived and is not likely to result in adverse health consequences (35). However, when the stressor is appraised as something that cannot be coped with, it will result in sustained arousal that is detrimental to one's health (10,11,13-16). Moreover, if we cannot, for some reason, adequately resolve the discrepancy in what was expected (*set values*) and the reality (*actual values*), the stress response, psychologically and physiologically, is continued (16). The stressor induces ongoing stress-related cognitions (e.g., worry). The perseverative cognition hypothesis states that these cognitions result in physiological activation for the duration of the cognition (17,18). These prolonged stress-related cognitions have been found to increase cardiovascular (CV) and hormonal activity in the laboratory and in real life (for a review see 21). However, in most studies, perseverative cognition explains only a part, or in some studies even none, of the prolonged CV responses to stressors (e.g., 21,23-25). Thus, although prolonged stress-related cognitions are important to evaluate as a factor in the etiology of somatic diseases, additional factors should be explored.

Psychological stress beyond self-report

In 1939 Alexander (36) suggested that unconscious processes may be involved in health-related processes. More specifically, his Specificity Theory entailed that unconscious conflicts could lead to somatic diseases such as hypertension. Although this psychoanalytic idea could not be verified with experimental studies, the idea that self-report does not fully capture associated threats to health is still an area of interest in psychosomatic medicine (for an overview see 28). Research on psychological stress relies mainly on self-report, but as mentioned above this approach has not yet led to identifying a sufficiently specified mechanism underlying the detrimental prolonged physiological responses to stressors (e.g., 15,21). Moreover, research suggests that as people may be unaware of their emotions, self-report questionnaires on affective states are likely to be insufficient (e.g., 37). Furthermore, there is evidence of stressors during the day leading to physiological activity during sleep, when there is no conscious cognition (e.g., 15,22,38), which suggests that factors beyond self-report may play an

important role. Hence, it has been proposed that people may not be aware of, or are unable to report on, part of their affective state (28), even when faced with health-relevant changes in the physiological state. This has been referred to as *unconscious stress* (26,27). It is suggested that stress-related cognitions may also occur outside of awareness. In fact, research on unconscious processes in other fields suggests a role of these processes in for example attitudes, self-esteem, emotions, central nervous system activity, decision making, and affective evaluation (39-47). Moreover, extending the reasoning of the CATS and PC hypothesis, these active representations of a stressor are hypothesized to simultaneously elicit physiological stress responses without the individual being aware of this process or even of the existence of these representations. This prevents active coping with the stressor, which may result in ongoing adverse physiological activation. In other words, even stressors that are not consciously noticed or cannot be reported on, may induce affective and bodily responses that may eventually lead to serious health problems. This is called the unconscious stress hypothesis, and forms the central hypothesis of this thesis.

The unconscious: A level of awareness

Psychology as a science has a long history of studying *the unconscious*. The repression theory of Freud has received considerable attention within and outside of the scientific community and referred to the unconscious as a different state of the mind that inhibited negative associations to prevent the conscious self from experiencing mental distress (see for a review 48). Experimental work was done in this area by for example Jung (1907, 49) and Peterson and Jung (1907, 50) (see also Chapter 2). Ever since these early studies, there has been an ongoing debate on the definition of consciousness (e.g., 51). Nowadays, an influential perspective is that consciousness should be thought of as a continuum in which multiple levels of awareness exist (40,43,51,52). According to Kihlstrom (1987, 53; 1993, 52) on one end of this continuum we have the mental states that are represented in phenomenal awareness, that is, conscious awareness. It can be assessed with questionnaires or introspection. The other end remains an enigma, with representations that are not available to phenomenal awareness, which is epitomized by the lack of a clear definition. For example, it has been defined as processes that occur while attention is directed elsewhere (in the case of decision-making; 42) and automatic activation (in the case of attitudes; 43).

Furthermore, different methodologies have been used to assess this end of the continuum of consciousness, such as subliminal presentation or implicit measures (53). Moreover, as suggested by for example LeDoux (1996, 54) and Wiens and Öhman (2010, 51) research on affective phenomena should not be limited to subjective indicators, because converging evidence from multiple indicators is much more persuasive of the existence of changes in the psychological state. However, in a large body of research on awareness not this theoretical notion of a continuum is conveyed, but

rather a dichotomy of unconsciousness versus consciousness (as elaborated on by for example Fazio & Olson, 2003, 43). Underlying this dichotomy lies the assumption that processes can be either conscious or unconscious, which, considering all evidence (e.g., 52,55,56), seems unlikely.

This thesis acknowledges that consciousness should be viewed as a continuum. Consequently, the operationalizations of unconscious processes are not meant to capture one end of a dichotomy and should be interpreted as addressing several levels of awareness other than what can be reported. Focusing on other levels of awareness beyond self-reports may help in explaining the variance in stress-related physiological responses. Moreover, although in the current thesis we have tried to address several levels of awareness with a range of methods chosen from the existing literature, it is not assumed that the realm of consciousness is limited to the levels on which these methods assess affective phenomena.

Inducing unconscious stress

To explore the role of unconscious processes in the relationship between stress-related information and (prolonged) physiological activation, we have taken two approaches; inducing and measuring unconscious stress.

Unconscious stress can be induced by activating the stress response outside of one's awareness, that is, by presenting a stressor below the threshold of awareness (i.e., subliminally). In experimental research, a common method to manipulate unconscious processes is subliminal priming, which has been shown to affect behavior, affect, and brain activity (e.g., 57-60), but also elicits CV changes (61-63). Even presentation times of 20 ms or shorter can elicit these effects (e.g., 64,65). The mechanism of subliminal priming relies on the activation of the associated evaluation of the prime once the prime is presented (66). The presented stimuli are usually sounds, images, and words (67). In the current context the associated evaluation of the prime should be stress-related. When presenting stress-related stimuli subliminally and measuring physiological responses, the unconscious stress hypothesis can be tested, that is, increased physiological responses to subliminal stress-related primes would provide evidence of unconscious stress. As discussed, psychological stress entails a range of negative affective cognitions, which can be specific (such as the word 'angry') but also nonspecific (such as a series of threatening words). However, the use of these stimuli requires the assumption that they elicit a certain level of psychological stress in all individuals, which is not necessarily true for each word and in each individual.

An alternative method that better ensures the induction of comparable stress-related cognitions to the same stimulus across participants is the use of a fear conditioning paradigm. This paradigm consists of pairing a stimulus with an aversive unconditional stimulus, such as a shock. The automatic physiological response to the aversive stimulus is thought to also occur in response to the (now) conditional

stimulus after repeated pairing (e.g., 45,68). The nonspecific state of vigilance and physiological activation that is induced by this method can be interpreted as a stress-response in line with learning-based stress theories (e.g., 16,69-72). In other words, by using a fear conditioning procedure, a (temporary) stressor can be created. By subsequently presenting the conditional stimulus subliminally and measuring the concurrent physiological changes, the unconscious stress hypothesis can be tested: Does the subliminally presented conditional stimulus (i.e., the stressor) increase physiological activity? In the past, increased autonomic nervous system activity to subliminally presented fear conditioned stimuli has been found (73), but no health-relevant parameters, such as blood pressure, have been addressed. In the current thesis, subliminal presentation of both previously validated stress-related stimuli and fear conditioned stimuli were used to induce unconscious stress.

Measuring unconscious stress

The second approach to testing the unconscious stress hypothesis is by measuring unconscious stress. Stress-related cognitions can be measured at different levels of awareness. In general, measures are designed to generate scores reflecting certain attributes of a person (74). To measure constructs outside of awareness implicit measures are applied. These measures use a procedure that prompts the subject to produce an automatic response that provides information about the assessed construct. Automatic in this context refers to "the absence of certain goals, awareness, substantial cognitive resources, or substantial time" (74). In this conceptualization of implicit measures it is stipulated that it is the measurement that is implicit and not the construct itself (see also 39,43). An implicit measure that is very prominent at present is the Implicit Association Test (IAT, 75). It was originally designed to measure implicit prejudices but has been adapted to suit other purposes and is increasingly used to measure implicit affect, referred to as the affective IAT. During an affective IAT, participants have to categorize stimulus-words that relate to the attribute of interest, for example *anxiety* vs. *calmness,* into target categories that relate to for example the *self* (e.g., me, I) or *others* (e.g., they, his) (76). The response index that is derived from the reaction times is thought to reflect the intensity of the attribute under investigation measured implicitly. Although debate is still ongoing concerning the correct interpretation and implementation, there seems to be consensus that these implicit measures do add to findings with self-report measures (75, 77-81).

Several studies have used implicit measures in relation to physiological measures during and after experimental stress inductions. For example higher negative affectivity measured with the IAT has been associated with higher BP and heart rate (HR) and lower heart rate variability (HRV; 76,82). In this thesis, other implicit measures that may be associated with the physiological correlates of psychological stress are evaluated. First, the Implicit Positive and Negative Affect Task (IPANAT; 83-85) was used, in which

participants have to indicate to which extent an artificial word has a certain affective value to them. Responses are thought to be a result of *affect infusion* (86), which means that representations of affective states at any level of awareness influence the affective value attributed to ambiguous stimuli. The responses are thought to reflect the activation of a certain affect. Previous studies have indicated that the negative affect subscale of the IPANAT was related to stressor-specific cortisol responses and higher levels of the cortisol awakening response (85,87). Regarding CV responses, correlational data have related the subscale to slower recovery of blood pressure after a stressor that was not found with the self-reported measure of affect (88). The possible practical and theoretical implications of these findings warrant further research.

Second, we used a Lexical Decision-making Task (LDT; 89), which is a common measure to capture *automatic vigilance*. In this task participants have to respond to a string of letters by indicating whether it represents a "word" or a "nonword". The actual words are positive, negative, or neutral in nature. Faster responses to a category indicate greater accessibility. It is thought that the affective representation that is activated enhances this accessibility, which leads to a quicker perception and processing of the presented stimuli of the category that is represented and is known as automatic vigilance (81). In earlier studies, automatic vigilance for negative information as measured with the LDT was found to be related to slowed recovery of HR (90). In the current thesis, these implicit measures are assumed to respond to induced psychological stress by increased negative affect on the IPANAT and shorter reaction times to negative words in the LDT.

These particular tasks were chosen for several reasons. First, the IPANAT and LDT use a series of emotion words covering the two ends of the spectrum of affect, negative and positive, rather than preset categories as in for example the IAT. In contrast to the IAT, both tasks are clinically applicable since the scores have a meaning at the individual level, and its administration procedure is straight-forward and brief. The IPANAT in particular directly assesses affect without having to extract it from affect-related self-concepts or attitudes (74,81,91). Importantly, these measures have the potential to be implemented on a mobile device for intervention purposes. Taken together these advantages and previous findings suggest that the IPANAT and LDT might be suitable candidates as implicit measure of psychological stress. Moreover, implementing measures that assess affective constructs such as psychological stress at an implicit level might provide the field with an additional tool to explain the occurrence, development, and progress of prolonged physiological activity that ultimately leads to (CV) disease. The explanatory value of these implicit measures, in addition to self-report measures, is explored in the current thesis.

Notably, in the studies where we tested if measures of unconscious stress could explain prolonged physiological activity after a stressful experience, we used stress-inductions to elicit the psychological and physiological stress response (92). In the

studies, we choose to administer two widely used stress-induction procedures. One was a mathematical task with anger harassment. The procedure was similar to that of a previous study (88), which found a relation between the negative subscale of the IPANAT and slower recovery of BP. However, that study lacked a control condition and firm conclusions regarding the IPANAT would be premature. The other procedure that we used was an anger recall task, which is a well-documented stress-induction task that is known to elicit CV responses (93).

Physiological correlates of (unconscious) stress

In the relationship between psychological stress and CV health, several outcome measures are of importance. CV activity can be expressed in various parameters that represent different aspects of the physiological state, but only those that are addressed in this thesis are described here. Blood pressure (BP) is most often expressed in SBP, the highest level of the pressure at the systole (i.e., contraction) of the heart, and DBP, the lowest level of the pressure at the diastole (i.e., relaxation) of the heart. Hypertension is diagnosed with a SBP of 140 mmHg or higher (160 mmHg when older than 60 years) and/or a DBP of 90 mmHg or higher (94). Mean arterial pressure (MAP) can be calculated from these two indices and is a more general vascular index that has been related to the baroreceptor activity (95). Heart rate (HR) is the number of heart beats per min (95). Heart rate variability (HRV) indicates the variability in timing of the heartbeats and fast fluctuations in this timing (especially when these occur within the frequency range of respiration) is thought to represent activity of the parasympathetic nervous system. In general, a higher HRV is considered healthy and is expected to be lower in response to stress-related stimuli (96). Furthermore, total peripheral resistance (TPR) is an index of resistance of the blood flow through the organism. Low TPR has been related to the development of CV disease and all-cause mortality (97,98). Additionally, TPR is thought to represent a specific dimension of the stress experience: A higher TPR would be associated with the experience of *threat* rather than *challenge* (99,100). Noninvasively it can only be calculated using other CV parameters (96).

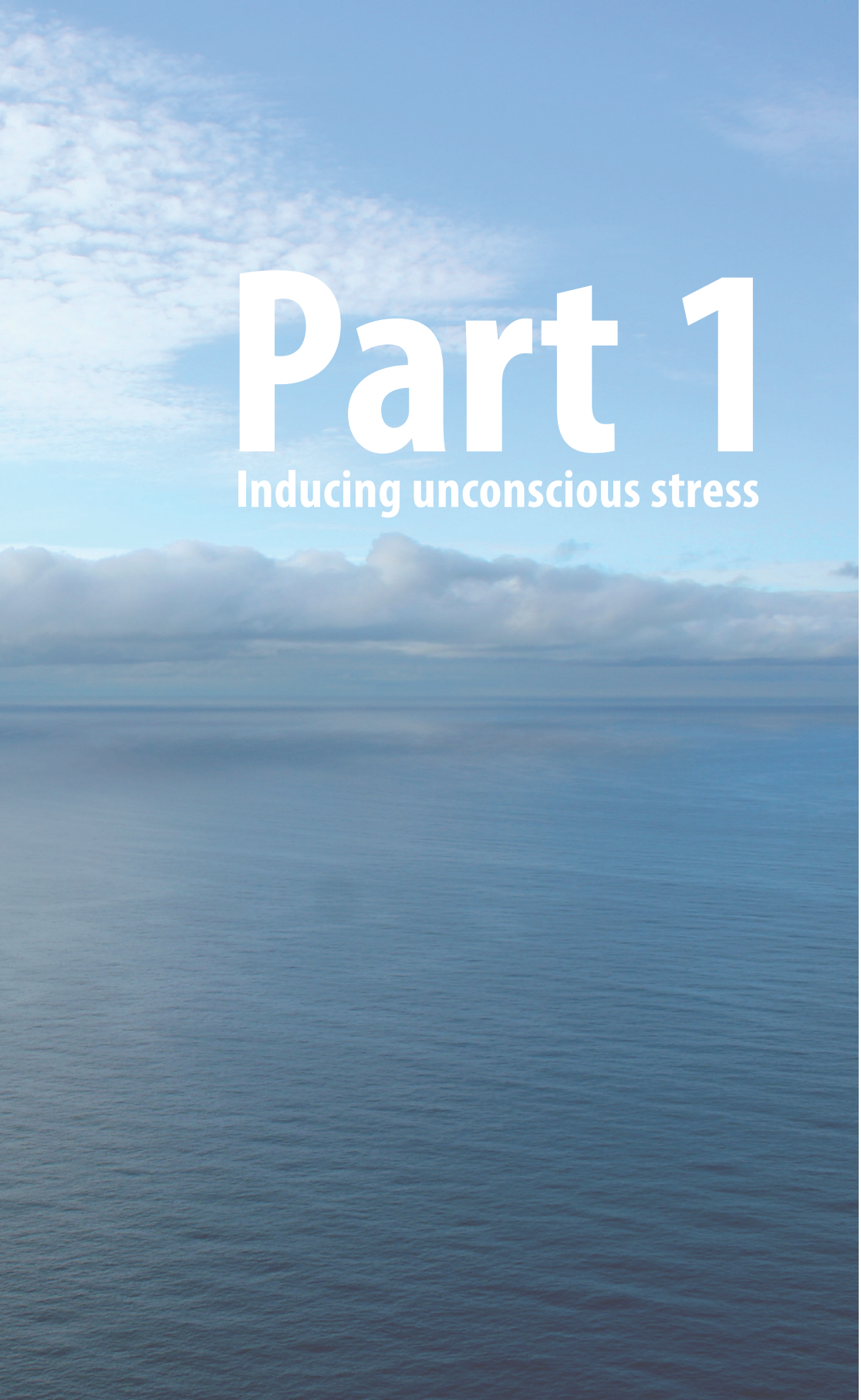
In the different experiments described in this thesis, we have used SBP, DBP, and HR to focus on immediate physiological changes, but in Chapters 3, 6, and 7 we looked at other indices of CV activity (MAP, TPR, and HRV) that help to more specifically clarify the specific state of the organism. All of these outcome measures have been described in relation to adverse consequences for health (e.g., 101,102). Furthermore, in the systematic review and the fear conditioning study we have also looked at skin conductance responses, as this is a common outcome measure in this research area (103), which we believe provides the necessary context for the outcomes of the study.

Main goals and outline

To sum up, by examining the CV responses to (subliminally presented) stressors and measuring psychological stress with self-report and implicit measures, this thesis will address whether unconscious stress can further explain the physiological response to a stressor.

In this thesis, a series of experimental studies is described using samples of healthy young adults, as an initial step to address the validity of the unconscious stress hypothesis. The central aim of these studies was to induce psychological stress and measure responses in physiological activity, but the methodology to address unconscious stress differed between studies. **Chapter 2** describes the systematic review of studies in which stress-related and stress-unrelated stimuli were presented subliminally, while peripheral physiological parameters were measured. In Chapters 3 and 4, we describe two studies in which we executed a subliminal priming paradigm on CV activity. More specifically, the study in **Chapter 3** tests the effect of subliminal threat words versus that of neutral words on CV activity. In **Chapter 4**, a study is described in which we presented the subliminal word 'angry' [woedend] and 'relax' [ontspan] to test the effects of this manipulation on CV activity. This study aimed to replicate the finding by Hull et al., (2002, 62), who observed that subliminally presenting the word 'angry' enhanced CV activity compared to the word 'relax'. However, as discussed above, the use of stress-related stimuli in subliminal priming paradigms offers an important limitation: it assumes pre-existing affective associations with the presented stimuli. However, these associations can differ greatly between individuals. In order to overcome this, we have tried to create a stressor with equal valence across participants in the study described in Chapter 5. In this study, we used a fear conditioning paradigm to create an association between neutral images and an aversive stimulus, a mild electrical shock. This association would create a physiological stress response that we expected to occur during a test phase in which these neutral images (CS+) were presented subliminally but without the shock, as compared with stimuli that were not paired with a shock (CS-).

In addition to these studies in which we looked at subliminally presented stress-related stimuli, we performed several experiments using measures beyond self-report after a stress-induction to assess the relationships with CV activity. In **Chapter 6** the Implicit Positive and Negative Affect Test was used after mental arithmetic that had to be performed with and without negative feedback. **Chapter 7** describes the associations of a Lexical Decision-making Task with CV responses after the recall of an anger or happiness evoking situation. In **Chapter 8** the findings of the studies are discussed to elaborate on the concept and challenges of the unconscious stress hypothesis. Together, these chapters provide a primary overarching approach in examining the relationship between psychological stress and health on the continuum of consciousness.



Part 1

Inducing unconscious stress

Chapter 2

Peripheral physiological responses to subliminally presented negative affective stimuli: A systematic review

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Abstract

Negative affective information may be presented outside of awareness and change physiological activity. By increasing peripheral physiological activity, subliminally presented negative affective information may contribute to the development of disease. The current systematic review evaluated 65 studies in which negative affective stimuli were presented subliminally to a healthy sample while cardiovascular, electrodermal, electromyographical, hormonal, or immunological activity was measured. Overall, 41% of the tested contrasts indicated significant increases due to negative affective stimuli compared to control stimuli. These effects were most pronounced in fear conditioning studies measuring skin conductance response amplitude and priming studies measuring systolic blood pressure. However, across the included studies the methodology varied substantially and the number of contrasts per physiological parameter was limited. Thus, although some evidence exists that subliminally presented negative affective stimuli can induce adverse peripheral physiological changes, this has not yet been addressed sufficiently.

Can information that occurs outside of awareness affect perception, motivation, decisions, and emotions? Research addressing this question is flourishing in various fields within psychology, including organizational (e.g., 81), emotion (e.g., 47), clinical (e.g., 59), cognitive (53), and social psychology (e.g., 66,104). Surprisingly, the potential role of unconscious processes in the relationship between negative affective information and health has remained understudied. In psychosomatic research, the limits of conscious awareness have long been of interest and explored (28). For example in the 1930s, a psychoanalytic approach was used to address unconscious emotional conflict in the etiology of hypertension (36), but experimental tests of this particular method failed to provide supportive evidence (28). Notwithstanding, the possible adverse influence of negative affective information outside of awareness on physiological systems is consistent with current theoretical insights (26-28,105,106). However, experimental evidence is still scarce. Given that several studies indeed showed that unconscious processes influence the experience of emotions (e.g., 107,108) and behavior (e.g., 57,109) it seems crucial to examine whether physiological parameters can be affected by negative affective stimuli when these are presented outside of awareness.

In fact, the quest for evidence of this kind appears to have a long history. In the early days of psychological research, Jung (1907, 49) and Peterson and Jung (1907, 50) performed several studies regarding the effect of word-associations on galvanic skin responses (GSRs). In these studies they would repeatedly read out a list of neutral words to participants that had to verbalize whatever associated word came to mind. The researchers observed that participants gave different verbal responses to some of the same words and, importantly, that the GSRs were larger than what they had seen before. Notably, this was one of the first psychophysiological experiments and not much was known about the electrodermal response at the time. An in-depth interview with the participants on these words revealed personal affective associations and that the changes in verbal responses had been unintentional. It was concluded that the GSR was able to detect affective associations with neutral words. The different verbal responses and GSRs together were assumed to be a new method to measure an attempt of the mind to prohibit further conscious processing of something that was considered harmful to the self and was referred to as the *psycho-physical galvanic reflex*. Although the authors faced considerable methodological restrictions using the electrodermal response, it seems that these findings are the first (published) displays of the physiological changes that involuntarily accompany an affective state. Later, McGinnies (1949, 110) was able to display negative affective words below threshold of awareness using a tachistoscope at an interval of 10 ms. He found larger GSRs to the affective words compared to the neutral words, which was interpreted as evidence for *perceptual defense*: a distortion of perception to protect the individual from unpleasant experiences. Moreover, Lazarus and McCleary (1951, 111) provided evidence that after a conditioning procedure individuals were able to discriminate between stimuli of

different affective valence before conscious recognition as indicated with changes in GSR, which was referred to as *subception*. Notably, the results of these studies have been largely discussed in light of the *repression hypothesis* as they were believed to indicate that individuals tend to reject and keep something out of consciousness when it may negatively affect one's wellbeing. These experimental researchers were pioneers and gave way to find ostensibly more objective evidence of physiological effects of subliminal negative affective information. The research instigated fierce criticism from peers, who performed what we would now call observational studies, and, as a result of the zeitgeist, may have been overlooked in their importance (for a historical discussion the reader is referred to MacKinnon and Dukes, 1962, 48).

More recently, influential evidence of the effects of subliminally presented negative affective stimuli on physiology is offered by neuroscience studies that have found amygdala activation in response to fear-inducing stimuli that were presented below threshold of awareness (e.g., 41,45,46). These findings suggest physiological arousal can be elicited using this type of stimulus presentation and support the earlier findings with GSR that differences in affective valence of stimuli can be determined even when these are presented outside of awareness. However, far less studies seem to have addressed peripheral physiological parameters, such as blood pressure or cortisol. Considering the potential relevance of unconscious processes in psychosomatic research, the aim of the current study was to provide a systematic review of the evidence for the physiological effects of subliminally presented negative affective stimuli from different fields within psychology.

This systematic review focused on studies that manipulated awareness of negative affective stimuli. In experimental designs, awareness is usually manipulated by presenting a stimulus below the threshold of awareness (i.e., subliminally) typically followed (and often preceded) by an irrelevant different stimulus (i.e., mask) (e.g., 51,112-114). Typically, this subliminal manipulation has been applied to two paradigms: priming with stimuli with an innate affective valence (e.g., 67), from here on referred to as 'priming studies', and priming with fear conditioned stimuli (e.g., 51), from here on referred to as 'fear conditioning studies'. The mechanism underlying the first paradigm, priming, is believed to be the activation of cognitive representations of the prime content, which is reflected in a change in a variety of behavioral responses such as reaction times to targets (66). In addition to behavioral responses, physiological responses have also been found to be influenced by subliminal affective primes (e.g., 62). In fear conditioning, an association between an unconditioned stimulus (US), such as a shock or a loud noise, that automatically elicits a response (i.e., unconditioned conditioned response, UCS) and a novel stimulus is formed. The result is a conditioned response (CR) to the now conditioned stimulus (CS+). In contrast, the stimuli that are not combined with a US are referred to as CS-. The participant is assumed to learn to differentiate between the CS+ and CS-. Presentation of the CS+ is expected to elicit a

physiological response that is similar to presentation of the US alone, as if it was the negative experience itself (e.g., 68). The advantage of fear conditioning over priming is that it offers more control over the specific affective associations with the stimulus.

Theoretically, the subliminal presentation of negative affective stimuli in experimental paradigms activates unconscious negative affectivity and should result in measurable changes in physiological activity (26-28). Since the dysregulation of adaptive peripheral physiological activity is assumed to be the final step in the relation between psychological negative affect and adverse health outcomes (e.g., 115), we only included studies using peripheral physiological parameters. Most of these parameters are believed to be more directly involved in increased somatic health risks than central nervous system parameters. For example stronger responses of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate variability (HRV) to mental stress were found to be predictive of cardiovascular (CV) disease risk and other health-related outcomes (e.g., 2,116,117). Furthermore, chronically elevated cortisol increases vulnerability for disease states, for example through immunosuppression and numerous other pathophysiological effects (118). As described, results generally confirm that subliminally presented stimuli affect the brain (e.g., 41,45,46), but this central activity does not necessarily provide information on peripheral activity. Moreover, findings regarding central activity have already been substantially elaborated on elsewhere (e.g., 40,119). In contrast, results on peripheral activity have scarcely been addressed and the potential health risks have not been evaluated. Thus, we focused on the peripheral physiological parameters that indicate physiological changes within the organism: CV and electrodermal (EDA) parameters of autonomic activity, musculoskeletal (i.e., electromyographical; EMG), hormonal, and immunological parameters. Additionally, by including only studies that tested a healthy population we attempted to elucidate the more general mechanisms that theoretically precede physical illnesses.

Searching the literature for research on the main concepts of this study, such as 'unconscious', is considerably hindered by a lack of consensus on terminology (see also 27,120,121). To overcome this issue we paid special attention to building a comprehensive keyword profile in an attempt to find all relevant studies. The complex method of building this profile is explained in detail in the method section. Basically, we systematically expanded an initial simple keyword profile with a large set of new keywords. Possible relevant keywords for 'unconscious' were for example alternatives such as 'subconscious' and 'without awareness'. A comprehensive and systematically built topic-specific profile increases the degree of certainty in finding all relevant articles. Moreover, it ensures replicability across databases and researchers while facilitating updates with exactly the same search profile over time.

Furthermore, we addressed two methodological issues regarding subliminal stimulus presentation. First, as pointed out by Eriksen (1960, 120) and Merikle (1984, 121), to obtain valid results regarding the effects of subliminally presented stimuli, a check of awareness of the presented stimuli is required to ensure that the stimuli are indeed not consciously perceived. Moreover, verbal report of awareness is subjective and objective measures of (non)awareness should be used (121). However, when recognition is reported using an objective measure, it implies that a participant has also consciously perceived (or processed) the stimulus, which is not necessarily true (122). To overcome this conundrum, we have extracted information on the type of awareness check without ascribing any value to the specific type of check. Second, changes in physiology after subliminal presentation of stimuli may be a consequence of the procedure itself, for example by seeing flashes on the screen or the use of masks that might have been arousing in some way. We addressed this by selecting studies with adequate control stimuli (i.e., stimuli that had no negative affective connotation) that were presented in the same way as the negative affective stimulus, either in between-groups or within-group designs.

Taken together, the primary research question of this systematic review is whether subliminally presented negative affective stimuli increase peripheral physiological activity compared with control stimuli. By providing an overview of studies regarding the role of nonconscious processes and potentially pathophysiologic mechanisms, this systematic review may add significant overarching knowledge about the effect of negative affective information on somatic health.

Methods

Keyword profile

We composed an elaborative keyword profile using BOOLEAN logic to formulate and combine the three sets of keywords pertaining to the three concepts: "unconscious", "negative affect", and "physiology". We started with a basic keyword profile in which the sets were separated by 'AND': *(unconscious* OR subconscious* OR nonconscious OR non-conscious OR preconscious OR pre-conscious OR sublimin* OR implicit*) AND (stress* OR arousal* OR (negative and (affect* OR emot*)) OR anxi* OR anger OR angr* OR fear OR threat*) AND (cortis* OR glucocort* OR adren* OR noradren* OR SCL* OR GSR* OR blood* OR blood-pressure OR systol* OR diastol* OR cardiac* OR heart* OR cardiovasc* OR immun*).* Subsequently, for each set we aimed to gather an exhaustive list of alternative keywords through the help of a native English speaker, the Thesaurus of PsycINFO, the synonym list of MS Word 2010, and previously found articles. For example in the case of the set "unconscious" we came up with 64 different conceptualizations, such as "nonconscious", "proprioception", and "repressed", see Table 1. Some keywords were

written differently across the articles and were thus formulated in all possible ways, for example "mindwandering", "mind-wandering", and "mind wandering". Instead of adding all keywords at once to the basic keyword profile each new keyword was added individually and its additional value was evaluated in terms of the number of new relevant articles found. This was established by searching the databases with a profile containing the new word and the two sets to which the word did not belong, while the set to which the new word did belong was "excluded" by using the NOT function of BOOLEAN logic. For instance in the case of the word "repressed" the evaluative profile would be: *repressed AND (*set keywords for "stress"*) AND (*set keywords for "physiology"*) NOT (*set keywords for "unconscious" without the new keyword*)*. This profile would yield *only* the articles that the keyword "repressed" added to the basic profile. When these articles were considered to be relevant, the keyword was added to its set in the basic profile. When the new keyword did not yield relevant articles it was not used anymore. The final profile that was build using this procedure is provided in Table 2.

TABLE 1 Keywords for "unconscious"

absence of awareness	latent inhibition	repressed
absent-minded	less conscious	represser
access dissociation	masked	repressing
affective stimuli	masked pictures	routinized
affective valence	masked stimuli	stimulus awareness
automatic processing	meta-consciousness	subconscious
automatic emotional	mind-wandering	subliminal
aware	non verbal	suboptimal
awareness	nonattended	suppressed
conscious awareness	nonconscious	suppresser
daydreaming	oblivious	suppressing
degree of awareness	outside of awareness	train of thought
emotional awareness	preattented	unaware
first order mental states	preattentive	unawareness
habitual	preconscious	unconscious
implicit	pre-cognition	unknowing
interoceptive awareness	precognitive	unnoticed unwanted thoughts
intuition	primary proces-level	unpremeditated
intuitive	prime	unwitting
involuntary	priming	without attention
lack of attention	proprioception	
latent	proprioceptive	

TABLE 2 Keyword profiles as inserted into the databases

Database	Web of Science	PsycINFO
Search details	Core Collection Advanced Search	Basic Search
Keyword profile	((TS=(unconscious* or subconscious* or nonconscious or non-conscious or preconscious or pre-conscious or sublimin* or implicit* or "automatic emotional" or "automatic emotion" or "automatic affect" or "automatic affective" or unattend* or mind-wandering or "emotional awareness" or "interoceptive awareness" or "degree of awareness" or "stimulus awareness" or "conscious awareness" or "involuntary stress" or "latent inhibition" or precogn* or pre-attent* or "automatic processing" or masked* or nonverbal or "non verbal communication") AND TS=(stress* or arousal* or (negative and (affect* or emot*)) or anxi* or anger or angr* or fear or threat* or ruminat* or worr* or "psychological tension" or shock* or "affective stimuli" or "priming" or "prime" or (emotional and (stimuli or circuit* or content* or state* or stimulation or expression))) AND TS= (cortis* or glucocort* or adren* or noradren* or SCL* or GSR* or blood* or blood-pressure or systol* or diastol* or cardiac* or heart* or cardiovasc* or immun* or "physiological arousal" or "physiological measures" or "physiological correlates" or "physiological activity" or "skin conductance" or autonomic* or EMG or (fac* AND (electromyography or muscle*))))) *AND* **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article)	(unconscious* or subconscious* or nonconscious or non-conscious or preconscious or pre-conscious or sublimin* or implicit* or "automatic emotional" or "automatic emotion" or "automatic affect" or "automatic affective" or unattend* or mind-wandering or "emotional awareness" or "interoceptive awareness" or "degree of awareness" or "stimulus awareness" or "conscious awareness" or "involuntary stress" or "latent inhibition" or precogn* or pre-attent* or "automatic processing" or masked* or nonverbal or "non verbal communication") AND (stress* or arousal* or (negative and (affect* or emot*)) or anxi* or anger or angr* or fear or threat* or ruminat* or worr* or "psychological tension" or shock* or "affective stimuli" or "priming" or "prime" or (emotional and (stimuli or circuit* or content* or state* or stimulation or expression))) AND (cortis* or glucocort* or adren* or noradren* or SCL* or GSR* or blood* or blood-pressure or systol* or diastol* or cardiac* or heart* or cardiovasc* or immun* or "physiological arousal" or "physiological measures" or "physiological correlates" or "physiological activity" or "skin conductance" or autonomic* or EMG or (fac* AND (electromyography or muscle*)))
Limiters	Indexes=SCI-EXPANDED, SSCI Timespan=All years	Peer-reviewed Human subjects

Search strategy

The procedures described by the PRISMA (Preferred reporting Items for Systematic Reviews and Meta-Analyses) Statement (123) were applied, to the extent that they apply to experimental research, to the literature search, data collection, and reporting of the results. The final keyword profile was used in Web of Knowledge (Core collection; field: 'topic') and PsycINFO (field: 'all text') on June 16, 2015. In Web of Science the search was limited to 'Article' as document type and 'English' as language. The used indexes were 'SCI-Expanded' and 'SSCI'. No limit to the time span was applied. In PsycINFO the limiters 'peer-reviewed' and 'human subjects' were applied. All duplicate publications were removed. For seven eligible articles the full-text could not be obtained through online methods; in one case we received the full-text version of the article from the authors, in two cases the authors were already deceased, and in the remaining four cases there was no response from the authors. The latter studies were discarded (124-127). Finally, we checked all references of the final selection of articles (i.e., a snowballing procedure) for articles that might not have been picked up by the keyword-profile. This resulted in ten possible new inclusions, of which three were eligible for inclusion. The databases were checked again for new articles on 16 December 2015 and resulted in one additional relevant article. Finally, one eligible article was accepted for publication at time of the second search and was obtained through personal communication.

Study selection and data collection

In total 2301 articles were evaluated for eligibility (See Figure 1). Articles were included when (1) subjects were healthy human adults, (2) an experimental design was used, (3) manipulation involved a negative affective stimulus, (4) the negative affective stimulus was manipulated out of the subject's awareness, i.e., processed without requiring conscious processing, (5) a control stimulus was used that was presented exactly like the negative affective stimulus for either between or within-group designs but was either of positive or neutral valence, (6) the dependent measure was a peripheral physiological outcome measure, (7) the article was peer-reviewed (e.g., no dissertations, conference proceedings, or editorials), (8) full-text was available in either English or Dutch.[1]

[1] The articles by Jung (1907, 49), Peterson and Jung (1907, 50), and McGinnies (1949, 110) were not included in the review. Although they are relevant in terms of the historical context of this systematic review, they were not found with our search strategy and did not meet the inclusion criteria. First, they were not selected using the keyword profile since the studies did not use a combination of the selected keywords. Furthermore, the articles did not include an abstract. Additionally, the snowballing procedure did not lead to inclusion of these articles. Moreover, the studies by Jung (1907, 49) and Peterson and Jung (1907, 50) did not include negative affective stimuli and the study by McGinnies (1949, 110) used 'critical words' as a manipulation, that would now probably be classified as high arousing rather than negative affective (raped, belly, whore, kotex, penis, filth, bitch). The studies would thus have been excluded for the review.

Eligibility was evaluated independently by two reviewers, the first and third author. A third reviewer, the second author, was consulted in case of disagreement. Articles that could not unanimously be excluded based on the information available at one step automatically were included in the next step to prevent invalid exclusion. The first round of exclusion was based on title; articles with titles that clearly implied an unrelated subject were discarded. After this round 679 articles were left. In the second round, exclusion was based on abstract and resulted in 184 potential eligible articles. Finally, in the third round the full-texts were evaluated which lead to the final inclusion of 54 articles. From articles that discussed multiple experiments studies that met the inclusion criteria were included as separate studies, resulting in a final selection of 65 studies.

The main features of the studies were extracted, as displayed in Table 3: Sample description, the nature of the negative affective stimulus, the key features of the design such as type of stimuli and presentation method, the type and data handling of the physiological parameters, awareness check, and the results. Data extraction was checked by at least one other author.

Quality assessment

To our knowledge no standardized quality assessment of experimental designs in psychology is available. To this end, we combined the Cochrane Collaboration's tool for assessing risk of bias (181) and the CAMARADES checklist (182) for quality of experimental animal studies. This resulted in a novel study quality assessment checklist of 15 items, of which 13 are applicable to experimental designs in psychology in general and two are specific for this systematic review. The items are displayed in Table 4.

The articles were awarded one point for each item, with a minimum score of 1 and maximum score of 15. The criteria were applied to articles and supplementary material. The findings are reported in Table 5 through 7 but are not further incorporated in decisions on eligibility or data collection since the assessment has not been used previously. However, the information was incorporated in the final conclusions regarding the research question.

Reporting

Performing a meta-analysis was not feasible due to the variety of employed stimuli and physiological parameters and differences in measurement procedures within studies using the same parameter (see Table 3). All results that addressed the current research question are reported based on the statistical significance as reported in the articles. Findings are summarized in terms of the specific *contrasts* that tested the effect of the negative affective stimulus compared with the control stimulus on the physiological parameters. The effect sizes were calculated and reported as η^2,η_p^2 or *d*, as appropriate (183). Furthermore, the nature of the awareness checks performed

in the studies are reported in Table 3. See Table 5 through 7 for the results per type of physiological parameter and type of experimental manipulation.

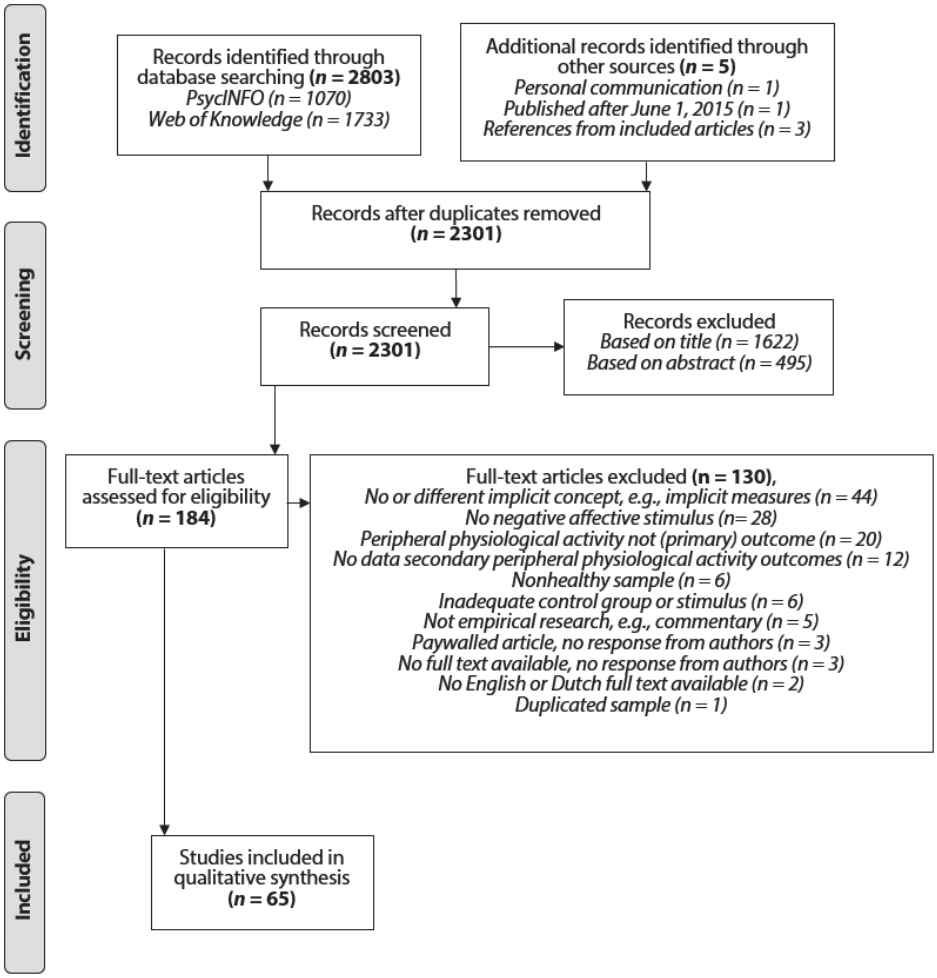

FIGURE 1 Flow chart of the selection process. Adapted from Moher et al. (2009, 123)

TABLE 3 Study descriptions split by type of manipulation (fear conditioning and priming)

Fear conditioning studies					
Author	*Year*	*Sample (age, N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Aderman et al.	1964	students, 18-25, 11	Subliminal extinction through variation in illumination on a projector	US: Shock after odd numbered stimuli, 75% reinforcement CS: Squares variating in size composed of parallel beams of white light projected onto the back of a milk-glass screen	Verbal report through individual threshold detection prior to study
Beisgen and Gibby	1969	volunteers, 19-25, 17	Subliminal acquisition and extinction through variation in exposure speed (CS: 50 - 200 ms)	US: Shock after CS+, 50% reinforcement CS: Set of nonsense syllables, five selected as CS+	Verbal report through individual threshold detection prior to study
Bunce et al.	1999	Males volunteers, 21.6 (1.4), 8	Subliminal acquisition through fast exposure (CS: 2 ms, CS-US 800 ms SOA)	US: Shock after CS+, 100% reinforcement CS: Schematic unpleasant face (CS+) and pleasant face (CS-)	Forced recognition task and visual threshold technique
Cornwell et al.	2007	volunteers, 18-48, 28	Subliminal acquisition (partial) through dichoptic configured images (CS: 50 ms, mask: 850 ms, on 50 ms, off 150 ms, alternating)	US: Shocks with unmasked presentation of CS+, 50% reinforcement CS: Red or green + or x sign Mask: Opponent colored stimulus	Verbal report
Corteen and Wood	1972	students, 19-34, 24	Dichotic listening in extinction through auditory masking (prose passage in one ear and target in the other)	US: Shocks after CS+ offset, 100% reinforcement CS: City names (CS+) and nouns (CS-) Mask: Prose passage	Verbal report
Flo et al.	2011	volunteers, 20-40, 16	Acquisition and extinction during S2 and REM sleep stages	US: Shocks or aversive images presented simultaneously with CS+, 100% reinforcement CS: Neutral sounds	N.A. (extinction during sleep)
Flykt et al. (study 1)	2007	students, 19-40, 64	Subliminal extinction through fast exposure and backward masking (CS: 25 ms, mask: 125 ms)	US: Shock or broad-band noise burst after CS+, 87.5% reinforcement CS: Snakes/guns away/towards the viewer Mask: Scrambled different images	Forced recognition task and verbal report
Flykt et al. (study 2)	2007	students, 18-44, 32	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 175 ms)	US: Shock or broad-band noise burst after CS+, 81.3% reinforcement CS: Snakes/guns away/towards subject Mask: Scrambled different images	Forced recognition task and verbal report

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Golkar and Öhman	2012	students, 24.9 (5.3), 27	Subliminal extinction through fast exposure and backward masking (CS: 33 ms, mask: 6 s)	US: Shock during CS+ 100%reinforcement CS: Fearful face images Mask: Neutral face images Other: Acoustic startle probe at six of the CSs	Forced recognition task
Lazarus and McCleary	1951	n.r., n.r., 9	Subliminal extinction through variation in 5 exposure times (CS: from 1/150 s to 1 s)	US: Shock after CS+ offset, 33.3% reinforcement CS: Nonsense syllables	Verbal report through individual threshold detection
Lipp et al.	2014	volunteers, 20.5 (2.44), 30	Binocular switch suppression through alternating the target and mask between visual fields with the target changing from black to full brightness at a rate of 2 Hz	US: Shock after CS+, 100% reinforcement CS: Image of snakes or wallabies Mask: Colored noise image	Signal detection experiment and verbal report
Núñez and De Vicente (study 1)	2004	students, 17-28, 36	Subliminal acquisition through fast exposure and backward masking (CS: individually determined, mask: 50 ms)	US: Shock after CS+, 100% reinforcement CS: The words 'magnesio' or 'locuacidad' Mask: XOXOXOXOXO	Individual threshold detection
Núñez and De Vicente (study 2)	2004	students, 17-25, 24	Subliminal acquisition through fast exposure and backward masking (CS: individually determined, mask: 50 ms)	US: Shock after CS+, 100% reinforcement CS: Two neutral words, two nonwords Mask: XOXOXOXOXO	Individual threshold detection
Öhman and Soares (study 2)	1993	students, 18-47, 64	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant (spider, snake), fear-irrelevant (flower, mushroom) images Mask: Scrambled different images	Forced recognition task
Öhman and Soares (study 3)	1993	students, 17-47, 32	Subliminal extinction through fast exposure and backward masking in alternating visual fields (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant (spider, snake), fear-irrelevant (flower, mushroom) images Mask: Scrambled different images	Forced recognition task
Öhman and Soares (study 1)	1998	students, 24.1 (n.r.), 40	Subliminal acquisition through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant or irrelevant images Mask: Scrambled different images	n.r.
Öhman and Soares (study 2)	1998	students, 19-39, 48	Subliminal acquisition and extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+ onset, 100% reinforced CS: Fear-relevant images Mask: Scrambled different images	Forced recognition task

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Olsson and Phelps	2004	students, n.r., 87	Subliminal acquisition and extinction through fast exposure and backward masking (CS: 33 ms, mask: 5973 ms)	US: Shock during CS+, unmasked trials only CS: Two angry male face images Mask: Neutral male face image	Verbal report
Parra et al. (study 1)	1997	students, 22.1 (n.r.), 24	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 30 ms)	US: Shock after CS+, 94,4% reinforcement CS: Angry, happy, and neutral male face images Mask: Face with similar valence but different from CS	Familiarity test
Parra et al. (study 2)	1997	students, 22.7 (n.r.), 40	Subliminal acquisition and extinction through fast exposure and backward masking (CS : 30 ms, mask: 30 ms)	US: Shock after CS+, 83.3% reinforcement CS: Angry or happy male face images Mask: Neutral face images	Familiarity test
Peper and Karcher	2001	volunteers, Group 1: 24.7 (5), 22	Subliminal acquisition and extinction through backward masking (CS: 30 ms, masks: in total 500 ms)	US: Baby cry (96 dB) after CS+, 71.4 % reinforcement CS: Face images without visual features but with positive or negative valence Mask: Scrambled facial elements	Forced recognition task
Saban and Hugdahl	1999	students, 18-28, 24	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Noise (90 dB) after CS+, 100% reinforcement CS: Two different male angry face images Mask: Neutral male face.	n.r.
Soares and Öhman	1993	volunteers, 18-47, 128	Subliminal extinction through backward masking (CS: 30 ms, mask: 100 ms)	US: Shocks during CS+, 83.3% reinforcement CS: Two fear-relevant or two neutral images Mask: Scrambled CS images	n.r.
Tassinary et al.	1984	students, n.r., 24	Subliminal extinction through fast exposure and dichoptic backward masking (CS: 10 ms nondominant eye, mask: 20 ms in dominant eye and 500 ms in both eyes)	US: Shock after CS+, 87.5% reinforcement CS: Names of body parts and animals Mask: Pattern mask (i.e., superimposed uppercase letters) Task: Lexical Decision Task[1]	n.r.
Wall and Guthrie	1959	students, n.r., 10	Subliminal extinction (partial) through fast exposure (CS: 1 s)	US: Shocks during CS+, 100% reinforcement CS: Neutral words Other: In testphase 1 CS visible in 50% of the trials	Verbal report
Wardlaw and Kroll	1976	n.r., n.r., 18	Dichotic listening in extinction through auditory masking (prose passage in one ear and target in the other)	US: Shocks after CS+, 100% reinforcement CS: City names (CS+) and nouns (CS-) Mask: Prose passage	Verbal report
Wiens et al.	2003	students, 18-28, 85	Subliminal acquisition through fast exposure and backward masking (CS: 10 ms, mask: 50 ms)	US: Shock after mask, group dependent reinforcement CS: Spider and snake images Mask: Scrambled CS images	Forced recognition task

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Wong et al.	1994	volunteers, 20.8 (1.4), 17	Subliminal extinction through energy masking with individually determined threshold	US: Shock after CS+, 83.3% reinforcement CS: Pleasant (CS-) and unpleasant (CS+) facial schematics Mask: Bright field with higher energy content than CS	Individual threshold detection
Worthington (study 1)	1966	students, n.r., 16	Subliminal acquisition through high illumination on a projector	US: Shock during CS+, 66.7% reinforcement CS: Words	Individual threshold detection and verbal report
Worthington (study 2)	1966	students, n.r., 16	Subliminal acquisition through high illumination on a projector	US: Shock during CS+, 66.7% reinforcement CS: Words	Individual threshold detection and verbal report
Priming studies					
Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Bornemann et al.	2012	students, 19.8 (1.39), 57	Subliminal exposure through fast exposure and backward masking (prime: 10/20 ms, mask: 2s)	NA prime: Angry face image Control primes: Happy and neutral face images Mask: Neutral face image or dotted pattern Task: Indicate valence of prime	Forced recognition task
Chatelain and Gendolla (study 1)	2015	students, 25, n.r., 42	Subliminal exposure through fast exposure and backward masking (prime: 27 ms, mask: 133 ms)	NA primes: Angry or fearful faces, 1/3 of the trials[2] Control prime: Happy faces, 1/3 of the trials Mask: Noise picture of black/white dots Task: Parity task[3]	Funneled debriefing
Codispoti et al. (study 1)	2009	students, n.r., > 42	Subliminal exposure through fast exposure (target: 25 and 80 ms)	NA prime: Unpleasant images Control primes: Pleasant and neutral images Task: None	n.r.
Codispoti et al. (study 2)	2009	students, n.r., 97	Subliminal exposure through fast exposure and backward masking (prime: 25, 40, 50 and 80 ms, mask: 1 s)	NA prime: Unpleasant images Control primes: Pleasant and neutral images Mask: Pattern mask Task: None	n.r.
Dimberg et al.	2000	students, n.r., 120	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 5 s)	NA prime: Angry face image Control primes: Happy and neutral face images Mask: Neutral face image Task: None	Pilot study and verbal report

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Garfinkel et al. (study 1)	2016	students, 22.7 (1.1), 18	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	n.r.
Garfinkel et al. (study 2)	2016	students, 24.6 (5.0), 14	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Forced recognition task
Gendolla and Silvestrini (study 1)	2011	students, 22 (n.r.), 45	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Angry or sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition task
Gendolla and Silvestrini (study 2)	2011	students, 21 (n.r.), 42	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Angry or sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition task
Hull et al. (study 3)	2002	students, n.r., 33	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Verbal report
Hull et al. (study 4)	2002	students, n.r., 64	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Verbal report
Jönsson and Sonnby-Borgström	2003	volunteers, 19-35, 53	Subliminal exposure through fast exposure and backward masking (prime: 17 and 56 ms, mask: 63 ms after prime offset)	NA prime: Angry male face images Control prime: Happy male face images Mask: Scrambled neutral face image Task: None	Written report
Kemp-Wheeler and Hill	1987	volunteers, G1: 19.7 (1.39); G2: 18-24, 28	Subliminal exposure through fast exposure and backward masking using dichoptic presentation	NA prime: Negative words Control words: Neutral words Mask: Pattern mask of scrambled numbers Task: None	Verbal report through individual threshold detection before experiment

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Kimura et al.	2004	male volunteers, 31.6 (6.4), 11	Subliminal exposure through fast exposure and dichoptic masking (prime: 30 ms, mask: 60 ms)	NA prime: Negative images Control prime: Neutral images Mask: Scramled prime images during prime presentation and flower images after prime presentation Task: Count amount of upside down flower images	Verbal report
Lapate et al.	2014	students, n.r., 46	Dichoptic presentation with emotional stimuli in the nondominant eye (1,023 ms) and mask in dominant eye (1,488 ms)	NA prime: Fearful face and spider images Control prime: Neutral face images Mask: Low contrast versions of prime Task: Indicate extend of liking the person based on a novel face	Forced recognition task and verbal report
Lasauskaite et al.	2013	female students, 20.5 (n.r.), 52	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 130 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face images Mask: Scattered black/white dots Task: Modified d2 mental concentration task[4]	None
Lasauskaite Schüpbach et al.	2014	students, 21 (n.r.), 134	Subliminal exposure through fast exposure and backward masking (prime: 27 ms, mask: 133 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face images Mask: Scattered black/white dots Task: Indicate correctness of an equation	n.r.
Lee and Tyrer	1981	students, 20-28, 48	Subliminal exposure through a neutral density filter of different transmission value in front of a projector lens and superimposed masking field	NA prime: Anxiety inducing motion picture Control prime: Neutral motion picture and motion picture with various abstract displays at two rates (18 ft/s and 24 ft/s) Mask: Neutral density filters reduced image intensity Task: None	Pilot study in different sample and verbal report
Najström and Jansson	2007	police recruits, 27.6 (2.88), 73	Subliminal exposure through fast exposure and backward masking (prime: 6 ms, mask: 200 ms)	NA prime: Unpleasant and arousing images (mostly physical threat) Control prime: Pleasant and calm images Mask: Scrambled prime image Task: None	Threshold detection task prior to study

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Nielsen and Kaszniak[5]	2006	paid volunteers, 21.2 (4.73), 17	Subliminal exposure through fast exposure and sandwich masking (forward mask: 45 ms, prime: 45 ms, backward mask: 2910 ms)	NA prime: Unpleasant and arousing images Control prime: Pleasant arousing images and neutral low arousing images Mask: Reassembled prime images Task: None	Forced recognition task
Ravaja et al.	2004	students, 19-35, 33-39	Subliminal exposure through six embedded facial expressions in a video clip (prime: 20 ms, mask: continuous)	NA prime: Angry face image Control primes: Happy or neutral face images Targets: Video clips of news items with different valence and arousal levels Mask: Face of newscaster Task: None	Verbal report
Reagh and Knight	2013	students, 18-24, 19	Subliminal exposure through fast exposure and backward masking (prime: 17 ms, mask: up to 8 s)	NA prime: Negative images Control primes: Positive and neutral images Mask: Random multicolored triangles Other: Startle probe white noise (100 dB) for 500 ms at 2, 4, and 6 s after trial onset during the mask on 72 trials Task: None	Forced recognition task and verbal report
Rotteveel et al. (study 2)	2001	students, 23 (5.9), 40	Subliminal exposure through fast exposure and backward masking (prime: 15 ms, mask: 2000 ms)	NA prime: Angry face images Control primes: Happy and neutral face images Mask: Chinese ideographs and neutral images Task: Indicate gender or valence of mask	Forced recognition task
Ruiz-Padial et al.	2011	female students, 20.4 (2.13), 35	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 100 ms)	NA prime: Unpleasant images Control primes: Neutral and pleasant images Mask: Unidentifiable display Other: Startle probe noise burst (105 dB) for 50 ms, between 3-4 s after stimulus onset in part of the trials Task: None	Forced recognition task and verbal report
Silvert et al.	2004	female students, 20-27, 17	Subliminal exposure through fast exposure and backward masking (prime: 29.4-47.1 ms, mask: 150 ms)	NA prime: Negative words Control primes: Neutral words Mask: Sequence of #s Task: None	Individual threshold detection

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Silvestrini and Gendolla	2011a	students, 23, n.r., 56	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition test
Silvestrini and Gendolla	2011b	students, 23 (n.r.), 75	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Sad face image, 1/3, 2/3, or 3/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition test
Smith	1993	students, n.r., 39	Subliminal exposure through editing a single frame of the prime into the video (prime: 16.7 ms, mask: continuous)	NA prime: Negative stimuli Control prime: Positive or neutral stimuli Mask: Neutral video Task: None	Verbal report
Sonnby-Borgström et al.	2003	students, 19-35, 61	Subliminal exposure through fast exposure and backward masking (prime: 17 and 53 ms, mask: 63 ms)	NA prime: Angry face image Control prime: Happy face image Mask: Nonfigurative grey-scale picture Task: None	n.r.
Sonnby-Borgström et al.	2008	volunteers, median 24, 100	Subliminal exposure through fast exposure and backward masking (prime: 17 - 23 or 65 - 70 ms, mask: 100 ms)	NA prime: Angry and sad face image Control prime: Happy face image Mask: N.r. Task: None	Pilot study and verbal report
Tan et al. (study 3)	2013	students, 21.6 (1.69), 36	Subliminal exposure through fast exposure and sandwich masking (forward mask: 133 ms, prime: 40 ms, backward mask: 133 ms)	NA prime: Negative images of animals or objects Control primes: Neutral images of animals or objects Mask: Abstract images Task: Respond to square	Forced recognition task
Weisbuch-Remington et al. (study 1)	2005	students, n.r., 107	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 1000 ms)	NA prime: Negative religious images Control prime: Positive religious images Mask: Same image reassembled and 180° rotated Task: Count number of tiles on mask	Verbal report

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Weisbuch-Remington et al. (study 2)	2005	students, n.r., 191	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 1000 ms)	NA prime: Negative religious and nonreligious images Control prime: Positive religious images and blurred religious images, 180° rotated Mask: Same image reassembled and 180° rotated Task: Count number of tiles on mask	Verbal report
Williams et al.	2006	volunteers, 35.8 (9.06), 15	Subliminal exposure through fast exposure and backward masking (prime: 16.7 ms, mask: 150 ms)	NA prime: Fear face image Control prime: Neutral face image Mask: Neutral face image Task: None	Forced recognition task and individual threshold detection
Williams et al.	2004	volunteers, 24.9 (7.5), 20	Subliminal exposure through fast exposure and backward masking (SOA: 10 ms and 30 ms, mask: 100 ms)	NA prime: Fear face image Control prime: Neutral face image Mask: Neutral face image Task: Indicate gender or age category of mask	Individual threshold detection

Note. The displayed sample descriptions are: the nature of the sample, age (*M* (*SD*) or range) and *N* (analyzed). *Abbreviations*: US = Unconditioned stimulus, CS = Conditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, ms = Milliseconds, SOA = Stimulus onset asynchrony, NA = Negative affective, n.r. = Not reported, N.A. = Not available, S2 = Stage 2 (sleep stage) REM = Rapid eye movement (sleep stage), s = Seconds, Hz = Hertz, dB = Decibel, G = Group, ft = Feet.

[1] In a Lexical Decision Task letter strings, or targets, are presented that form words or nonwords that have to be categorized accordingly. The primes precede these strings (e.g., 67).

[2] In the remainder of the trials a neutral face image was used as a prime.

[3] Parity task: Paradigm to test attentional engagement to emotional stimuli (179).

[4] In this study participants had to discriminate between two or other amount of apostrophes around p or d, which is a modified version from the original d2 test of attention (180).

[5] The study compares meditators and controls, but for the current review the data for the healthy controls were extracted

TABLE 4 Description of the criteria used for the quality assessment of the studies

Criterion	Description
1.	Publication in peer-reviewed journal
2.	Complete sample description (indication of age and nature of sample)
3.	Reports sample size and number of drop-outs
4.	Randomized allocation of participants to groups in between-subjects designs and randomized presentation of stimuli across trials in within-subject designs
5.	Inclusion of a control group in between-subjects designs, or control trials or phases in within-subject designs
6.	Blinded researcher
7	Blinded allocation or outcome concealment
8.	Ethical aspects addressed by a review committee
9.	Report Mean and Standard Deviation or Standard Error of the Mean and/or effect size
10.	Report outcomes of statistical tests (*F*/*T* ratios or regression coefficients)
11.	Report significant and nonsignificant results
12.	Report outcomes of all dependent variables described in method section
13.	Statement on possible conflict of interest
14.	Included an awareness check
15.	Description of method of physiological data handling

Results

Nature of the included studies

A summary of the results can be found in Figure 2. Importantly, the literature search did not reveal any studies with hormonal or immunological parameters and results are thus restricted to the CV, EDA, and EMG parameters. Furthermore, the search was not limited to specific experimental methods, but only articles using a fear conditioning or priming paradigm were found. Finally, the study samples usually consisted of students, but in several studies other groups were included such as unpaid and paid nonstudent volunteers, police recruits (149), and meditators versus nonmeditators (150).

Fear conditioning studies. Thirty studies used fear conditioning to induce a negative affective state. These experiments consist of an acquisition phase, during which the CR is created, and an extinction phase, during which the occurrence of the CR in absence of the US is observed (e.g., 68). In both phases stimuli can be presented subliminally. The CR to a CS+ is compared with responses to the CS-. CRs were only considered, in both the acquisition and extinction phase, when the US was not presented or presented outside the time-window during which the CR was measured. The CS+ usually consisted of fear-relevant images (e.g., spiders), or neutral stimuli (e.g., neutral shapes or sounds).

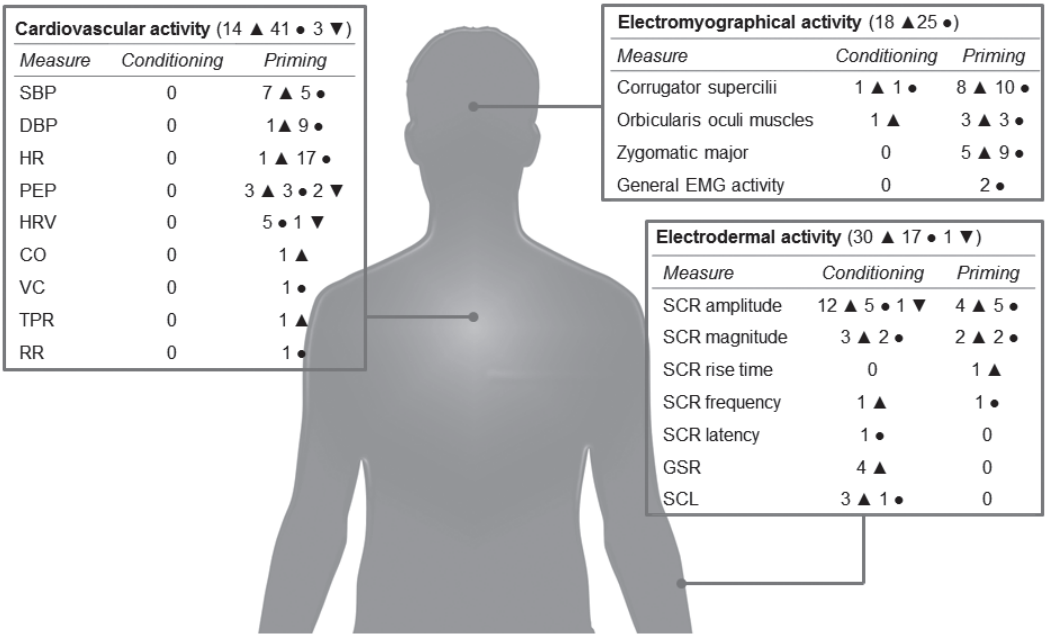

FIGURE 2 Schematic summary of the results expressed in the number of tested contrasts for each type of outcome measure per type of study. The direction of the results are indicated with ▲(NA stimuli > control stimuli), ▼(NA stimuli < control stimuli), and ● (no difference between the stimulus types). *Abbreviations*: SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, PEP = Pre-ejection period, HRV = Heart rate variability, CO = Cardiac output, VC = Ventricular contractility, TPR = Total peripheral resistance, RR = Respiratory rate, GSR = Galvanic skin response, SCR = Skin conductance response, SCL = Skin conductance level

Subliminal presentation was achieved through different procedures: fast presentation with and without backward masking, brightness differences (i.e., illumination), dichoptic presentation (i.e., presentation of different stimuli to each eye), and dichotic listening (i.e., presentation of different stimuli to each ear). Less common techniques to induce the CRs involved procedures during sleep, binocular switch suppression (i.e., intermittently switching the stimulus and mask between both eyes), and energy masking (i.e., extremely limited stimulus exposure before a blank field of equal luminance). As awareness check, the studies generally used verbal report, but several studies used more objective methods such as a threshold detection task and forced recognition of the stimuli. Some studies did not report an awareness check (13.3%) and in one study this was deemed unnecessary since the participants were asleep during the experiment.

Priming studies. Thirty-five studies used subliminal priming to induce a negative affective state. In these experiments affective stimuli, referred to as 'primes', are

presented during an often irrelevant task. Responses are compared with those to positive or neutral (i.e., control) stimuli. In most studies, the negative affective primes were images of faces displaying a negative emotion (anger, sadness, or fear), but also other unpleasant images, negative words, or an anxiety inducing motion picture.

In most priming studies, subliminal presentation was achieved through fast presentation with backward masking, 'sandwich masking' (i.e., presenting a mask before and after the prime), or fast exposure without masking. Some studies embedded the stimuli into a video clip, used dichoptic presentation, or placed a neutral density filter in front of a projector lens. Generally, verbal report was used to check whether participants had been aware of the stimuli. Other methods were a pilot study, individual threshold detection tasks, forced recognition tasks, or a funneled debriefing. Not all studies (15.2%) reported an awareness check and one study reported that an awareness check was not performed.

Findings of the included studies

Electrodermal activity (EDA). EDA measures are based on several different quantifications of skin conductance recordings (for a description of the specific measures the reader is referred to 103). The findings are displayed in Table 5 and operationalizations of the EDA measure are reported for each study.

Fear conditioning studies. In 23 fear conditioning studies, EDA responses were measured to test 33 contrasts that compared subliminally presented negative affective stimuli (CS+) with control stimuli (CS-). In 12 out of the 18 contrasts measuring s*kin conductance response* (SCR) *amplitude* a larger response to the CS+ compared to the CS- was found, while five contrasts yielded no difference between the CS+ and CS-. Furthermore, one study found a reversed effect, that is, the responses to the CS+ were smaller compared with the CS-. Several other EDA measures were used; *SCR magnitude* was higher in three contrasts but lower in two other contrasts, the number of *galvanic skin responses* (GSRs) was larger in four contrasts, changes in *skin conductance level* (SCL) were larger in three contrasts and equal in one contrast, *SCR frequency* was higher in one contrast, and, finally, no changes were found for *SCR latency* as tested by one contrast.

Importantly, a higher SCR amplitude was generally found in response to fear-relevant CS+ as opposed to fear-irrelevant CS+ that elicited a higher SCR amplitude. Regarding the other EDA parameters, most studies used fear-relevant stimuli, but did not compare them to fear-irrelevant stimuli. Only Lipp, Kempnich, Jee, and Arnold (2014, 148) made this comparison, by using snakes (fear-relevant) and wallabies (fear-irrelevant) as conditioned stimuli, and did not find a difference in the responses.

Priming studies. The majority of the 10 priming studies, testing 15 contrasts, measured *SCR amplitude*. Four contrasts showed higher SCR amplitude in response to the subliminally presented negative affective stimuli, but five contrasts yielded

no differences. With respect to the other EDA parameters, two contrasts yielded a greater *SCR magnitude* after the negative affective stimuli compared to the control stimuli, whereas two contrasts did not. Furthermore, one contrast yielded a higher *SCR rise time* after the negative affective stimuli compared to the control stimuli. Finally, another contrast showed that the mean *SCR frequency* was equal after both stimulus types.

Conclusion and recommendations. All in all, in 30 of the 48 (63%) contrasts EDA responses increased to subliminally presented negative affective stimuli relative to the control stimuli. These significant differences were mostly found in studies using SCR amplitude as the EDA parameter, fear conditioning as the experimental paradigm, and fear-relevant stimuli. The inconsistencies in the results can be attributed to several factors. In general, a great variation is apparent between studies in determination of the time window of interest, apparatus, and statistical data transformations. For example when determining SCR amplitude, individual differences should be taken into account by applying a range correction should be applied (184). This is a procedure in which the SCR of the individuals is expressed in the maximum and minimum level of response amplitude. However, not all studies performed this correction. Additionally, there are substantial differences in the quantification of the various EDA responses (103). For example GSR was expressed in number of responses but also in amount of (in)correct responses, and the time window in which SCR is expected to increase compared to a baseline was different across studies (see Table 5). Furthermore, room temperature and humidity have been found to affect EDA. For example SCR amplitude can increase with a 1° increase in room temperature (for an overview see 185). However, none of the studies reported on these factors. Finally, not all studies reported on the handling of nonresponders. Since part of the general population might not show EDA responses (e.g., 186), it should be explicitly addressed whether the data of these nonresponders were omitted or not. This was generally not the case in the included studies.

In conclusion, the reviewed literature suggests that negative affective stimuli that are presented below threshold of awareness might increase EDA responses compared with control stimuli, but this effect might be limited to stimuli that are 'biologically fear-relevant' (187), such as snakes and spiders.

TABLE 5 Study outcomes for electrodermal responses (i.e., galvanic skin response (GSR), Skin conductance level (SCL), Skin conductance response (SCR)) per manipulation type

Fear conditioning studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Aderman et al.	1964	GSR: Number of correct and incorrect responses based on substantial deflection[2]	CS+ > CS-	-	9
Beisgen and Gibby	1969	GSR: Correct and incorrect responses	CS+ ≠ CS-	-	9
Cornwell et al.	2007	SCR: Magnitude, max. deflection initiated at 500-5000 ms after onset of stimulus > 0.03 µS, averaged per CS	CS+ = CS-	-	13
Corteen and Wood	1972	SCL: Number of responses based on change > 1 K ohm, within 3 s after CS	CS+ > CS-	-	9
Flo et al.	2011	SCL: Change > 0.03 µS within 1 - 5 s after CS, with a subsequent reduction in mV of 1/3 peak value	Across groups[3]: S2 (extinction): CS+ > CS- REM (extinction): CS+ > CS-	η_p^2 = 0.28 η_p^2 = 0.22	7
Flykt et al. (study 1)	2007	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS onset > 0.032 µS. Applied a range correction[4]	Snakes: CS+ > CS- Guns: CS+ > CS-	*d* = 0.30 *d* = 0.18	12
Flykt et al. (study 2)	2007	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS onset, > 0.032 µS. Applied a range correction	CS+ = CS-	-	12
Lazarus and Mc Cleary	1951	SCR: Amplitude, averages of GSR's over 5 s after CS	CS+ > CS-	*d* = 2.48	7
Lipp et al.	2014	SCR: Magnitude, max. response initiated within 4 - 7 s after CS onset, averaged across the four trials per CS+ and CS- in each block	CS+ > CS-[5]	η_p^2 = 0.43	11
Núñez and De Vicente (study 1)	2004	SCR: Amplitude, within 4 s after the CS+. Applied a range correction	n.r.	-	8
Núñez and De Vicente (study 2)	2004	SCR: Amplitude, within 4 s after the CS+. Applied a range correction	n.r.	-	8
Öhman and Soares (study 2)	1993	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant: CS+ > CS- Fear-irrelevant: CS+ = CS-	*d* = 0.66 -	11

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Öhman and Soares (study 3)	1993	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant: CS+ > CS- Fear-irrelevant: CS+ = CS-	d = 0.94 -	11
Öhman and Soares (study 1)	1998	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Acquisition: CS+ > CS-, in fear-relevant group only	d = 1.44	10
Öhman and Soares (study 2)	1998	SCR: Magnitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Averaged per block of four trials. Square root transformed. Applied a range correction	In all groups[6]: Acquisition: CS+ > CS- Extinction: n.r.	η_p^2 = 0.58	12
Olsson and Phelps	2004	SCR: Peak-to-peak amplitude difference to the first response within 0.5 - 4.5 s after CS onset, > 0.02 µS. Square root transformed	In all groups: Acquisition: CS+ > CS-, in Pavlovian group only Extinction[7]: CS+ = CS-	- -	12
Parra et al. (study 1)	1997	SCR: Amplitude of the largest response measured initiated within 1 - 4 s after CS onset on the unreinforced trials. Applied a range correction	CS+ > CS-	d = 0.40	10
Parra et al. (study 2)	1997	SCR: Amplitude of the largest response measured initiated within 1 - 4 s after CS onset on the unreinforced trials. Applied a range correction	Acquisition: CS+ > CS- Extinction: CS+ > CS-	η_p^2 = 0.38 d = 0.40	11
Peper and Karcher	2001	SCR: Amplitude, max. deflection within 1 - 4 s after stimulus onset, > 0.05 µS, Log (+1) transformed. Applied a range correction	n.r.	-	10
Saban and Hugdahl	1999	SCR: Amplitude, responses within 1 - 4 s after CS onset, > 0.004 µS during 100 ms epochs. Applied a range correction.	n.r.	-	10
Soares and Öhman	1993	SCR: Amplitude, max. deflection initiated within 1 – 4 s after a CS, > 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant CS: CS+ > CS- Neutral CS: CS+ = CS-	d = 0.71 d = 0.61[8] -	9

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Tassinary et al.	1984	SCR: Amplitude, within 1.5 s prior to CS onset, ending 4 s later. Phasic response scores were converted into z-scores	CS+ < CS-	η_p^2 = 0.47	7
Wall and Guthrie	1959	GSR: 10 stimulus presentations coded + or - relative to their median GSR	CS+ > CS-	-	5
Wardlaw and Kroll	1976	SCL: A response occurred with a change of at least 1 K ohm, within 3 s after CS	CS+ = CS-	-	10
Wiens et al.	2003	SCR: Magnitude of first response initiated within 0.9 - 4 s after CS onset. Square root transformed	CS+ > CS[9]	η_p^2 = 0.26	10
Wong et al.	1994	SCR: Change in SCL (delta C) > 0.1 µmho between prestimulus level (400 ms) and within 1 - 4 s after CS. Square root transformed	Frequency: CS+ > CS- Amplitude: CS+ > CS- Latency: CS+ = CS- Magnitude: CS+ = CS-	*d* = 0.45 *d* = 0.39 - -	9
Worthington (study 1)	1966	GSR: Mean % changes (changes within 1 - 4 s after CS onset) of first and last 6 unreinforced CS presentations	n.r.	-	10
Worthington (study 2)	1966	GSR: Mean % changes (changes within 1 - 4 s after CS onset) of first and last 6 unreinforced CS presentations	Acquisition: CS+ > CS-	*d* = 1.13	10
Priming studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Codispoti et al. (study 1)	2009	SCR: Amplitude, max. change within 1 - 4 s after stimulus onset. Log transformed	Unpleasant = pleasant[10]	η^2 = 0.13	7
Codispoti et al. (study 2)	2009	SCR: Amplitude, max. change within 1 - 4 s after stimulus onset. Log transformed	< 80 ms[11]: Unpleasant = neutral and pleasant	-	7
Kimura et al.	2004	SCR: Amplitude, max. deflection within 1 - 4s after stimulus offset, > 0.05 µS. Log transformed (+ 1)	n.r.	-	11
Lapate et al.	2014	SCL: Amplitude, change within 1- 4 s after stimulus onset, > 0.02 µS. Square root transformed	Fearful faces > neutral Spiders = neutral	*d* = 0.32 *d* = 0.19	13

Author	*Year*	*Units of analysis*	*Results*	*Effect size*	*Quality (1-15)*
Lee and Tyrer	1981	SCL: Amplitude, n.r.	n.r.	-	11
Najström and Jansson	2007	SCL: Amplitude, distance between lowest and highest response within 1 s before and 11 after stimulus presentation. Square root transformed	Threat > neutral	$d = 0.22$	12
Nielsen and Kaszniak[12]	2006	SCR: Magnitude, first SCR within 1 - 4 s after stimulus onset, > 0.03 µS	Threat = neutral Threat = pleasant	-	11
Reagh and Knight	2013	SCR: Amplitude, max. response during 10 s after startle presentation compared to baseline (response onset), > 0.05 µS	Negative > neutral Negative > happy	$d = 1.15$ $d = 1.16$	12
Silvert et al.	2004	SCL: Magnitude, changes within 1 - 4 s after stimulus onset, averaged for each type of word. Square root transformed	Negative > neutral	$d = 0.65$	11
Tan et al. (study 3)	2013	SCR: Magnitude, max. change during 1 – 5 s after stimulus onset. Log transformed (+ 1)	Negative > neutral	$\eta^2 = 0.16$	13
Williams et al.	2006	SCR: Amplitude, change within 1 - 3 s after stimulus offset compared with baseline, > 0.05 µS	Fear = neutral	$d = 0.49$	12
Williams et al.	2004	SCR: Amplitude, change within 1 – 3 s after target offset compared with baseline, > 0.05 µS	Amplitude: Fear = neutral Frequency: Fear = neutral Rise time: Fear > neutral[13]	- - $d = 0.49$, 0.66	9

Note. *Abbreviations*: GSR = Galvanic skin response, CS = Conditioned stimulus, US = Unconditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, SCR = Skin conductance response, ms = Milliseconds, µS = MicroSiemens, SCL = Skin conductance level, K ohm = Kilo ohm (resistance), s = Seconds, mV = Millivolt, S2 = Stage 2 (sleep stage), REM = Rapid eye movement (sleep stage), n.r. = Not reported, µrho = Microrho (density).

[1] Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, but are not always available due to missing information (e.g., cell sizes).

[2] Deflection is a common used term for 'change in SCL' (185).

[3] Group 1: US: Images with negative emotional valence, CS: Neutral sound; Group 2: US: Mild electric shock, CS: Neutral sound; Group 3: Aversive shocks during sleep.

[4] Range correction: For each individual the SCRs were divided by the largest response amplitude recorded during the experiment to reduce irrelevant variation caused by differences in reactivity between subjects (184).

[5] Independent of the nature (snakes or wallabies) of the CS+.

Continues >

Electromyographical (EMG) activity. Surface EMG measures the electromagnetic field at the surface of the skin (for a description of the outcome measures the reader is referred to 188). The findings are displayed in Table 6 and operationalizations of the EMG measure are reported for each study.

Fear conditioning studies. In three fear conditioning studies EMG was measured and three contrasts were tested. One contrast yielded a higher response of the *corrugator supercilii* to the CS+ compared to the CS-. Another contrast did not find this difference, but in that specific study the exposure time to the target was exceptionally low (2 ms; 131). Furthermore, one contrast showed a higher *orbicularis oculi* response to the CS+ compared to the CS-.

Priming studies. In 13 priming studies, testing 40 contrasts, EMG was measured. Where eight contrasts found higher activity of the *corrugator supercilii* in response to negative affective stimuli compared to the control stimuli, ten other contrasts did not find this difference. A similar pattern was found for *zygomatic major* activity as for the corrugator supercilii but reversed (as expected): five contrasts indicated lower zygomatic major activity in response to the negative affective stimuli compared to the control stimuli, whereas nine contrasts did not find a difference. Furthermore, three contrasts testing *orbicularis oculi muscles* activity showed a higher peak response to negative affective stimuli compared to the control stimuli, but three other contrasts did not indicate any differences between stimulus types. Finally, two contrasts indicated that *general EMG activity* was not different between the stimulus types. Importantly, Codispoti, Mazzetti, and Bradley (2009, 133) found a significant difference in *corrugator supercilii* activity at a stimulus presentation of 50 ms, but not at faster presentation times (i.e., 25 ms and 40 ms). Additionally, Sonnby-Borgström, Jönsson, and Svensson (2008, 168) found an effect of presentation duration on the *zygomatic major* activity; negative affective stimuli elicited a smaller response than the control stimuli, but only when presented for 17-23 ms and not for 65-70 ms.

[6] Groups differed in received instructions on the goal of the study, but the overall pattern was the same.

[7] Groups comprised of either Pavlovian learning, observational learning, or instructed learning.

[8] At the start of extinction half of the subjects were informed that no more shocks would be administered and other half was not informed. Effect size is reported for these respective groups.

[9] Omnibus analysis of variance that includes the supraliminal condition. A significant interaction was found for condition, $F(3,81) = 9.50$, but the main effect of condition was not significant, $F < 1$.

[10] Exposure durations: 25 ms, 80 ms, 250 ms, 500 ms, 1500 ms, 2000 ms, 5000 ms, and 6000 ms.

[11] Exposure durations: 25 ms, 40 ms, 50 ms, 80 ms, 150 ms, 250 ms, and 1000 ms.

[12] Results are reported for the control group only.

[13] Exposure durations: 30 ms and 10 ms are reported, respectively

Conclusion and recommendations. Together, 18 of the 41 contrasts (44%) reported hypothesis congruent results for the measured EMG response (i.e., increases to subliminally presented negative affective stimuli compared with control stimuli). Although the results for the different types of EMG measures are mixed, the findings point toward an increase of EMG activity after subliminal negative affective stimuli (or a decrease in the case of smiling). There are some possible explanations for the mixed results. One main issue in EMG measurement is the relatively subtle signals it produces and its high susceptibility to noise from task-unrelated sources. Sufficient noise reduction, attention to electrode sizes, and appropriate signal conditioning techniques should be employed (188). In the reviewed literature, most of the studies corrected for noise and reported details on measurements and data reduction. However, some studies merely reported that "the measurement had been performed and analyzed" (165,170). Furthermore, zero signal baselines are hard to obtain for EMG measures since muscle activity is rarely absent. More importantly, placing electrodes on facial muscles might already influence participants to show unnatural responses (188). In sum, from the current literature it cannot be concluded that negative affective stimuli below threshold of awareness differentially influence EMG compared with control stimuli.

TABLE 6 Study outcomes for electromyographical measures per manipulation type

Fear conditioning studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Bunce et al.	1999	Corrugator supercilii: Mean change from baseline (400 ms before CS onset) in response to stimulus. Log transformed	CS+ = CS-	$d = 0.04$	12
Golkar and Öhman	2012	Startle: Mean startle magnitude to startle probe between trials	CS+ > CS-	$\eta^2 = 0.44$	12
Tassinary et al.	1984	Corrugator supercilii: Averaged over 0.5 s intervals from 1.5 s prior to CS and 4 s after,	CS+ > CS-	$\eta_p^2 = 0.47$	7
Priming studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Bornemann et al.	2012	EMG activity: Mean change from baseline (200 ms before prime onset) over a 2 s interval	Zygomatic major: Anger = happy Anger = neutral Corrugator supercilii: Anger > happy Anger > neutral	 $d = 0.13$ $d = 0.06$ $d = 0.55$ $d = 0.37$	8
Codispoti et al. (study 1)	2009	Corrugator supercilii: Mean change from baseline (1 s before prime onset) over a 6 s interval	Unpleasant > neutral & pleasant, at all exposure durations[2]	$\eta^2 = 0.39$	7
Codispoti et al. (study 2)	2009	Corrugator supercilii: Mean change from baseline (1 s before prime onset) over a 6 s interval	25 & 40 ms[3]: Unpleasant = neutral & pleasant 50 ms: Unpleasant > pleasant Unpleasant > neutral	- $\eta^2 = 0.08$ $\eta^2 = 0.08$	7
Dimberg et al.	2000	EMG activity: Mean change from baseline (1 s before prime onset) averaged over 100 ms epochs during the first s of exposure	Zygomatic major: Anger < happy Anger < neutral Corrugator supercilii: Anger > happy Anger > neutral	 $d = 0.68$ $d = 0.36$ $d = 0.56$ $d = 0.37$	10
Lee and Tyrer	1981	Frontalis: N.r.	n.r.	-	11
Nielsen and Kaszniak[4]	2006	EMG activity: Mean change from baseline (1 s before prime onset) of 100 ms epochs during stimulus presentation (3 s)	Zygomaticus major: Unpleasant = neutral Unpleasant = pleasant Corrugator supercilii: Unpleasant = neutral Unpleasant = pleasant	-	11

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Ravaja et al.	2004	EMG activity: Mean values of each of the first three 15-s epochs during the stimulus presentation	Zygomaticus major: Anger = happy Anger = neutral Corrugator supercilii: Anger = happy Anger = neutral Startle: Anger = happy Anger = neutral	 *d* = 0.13 *d* = 0.07 *d* = 0.08 *d* = 0.08 *d* = 0.13 *d* = 0.08	14
Reagh and Knight	2013	Startle: Mean peak response from baseline (50 ms before to 20 ms after startle probe onset) between 20 and 150 ms after startle probe onset	Negative > neutral Negative > happy	*d* = 0.95 *d* = 1.00	12
Rotteveel et al. (study 2)	2001	EMG activity: Mean over first 500 ms prior to trial onset and 3 s following prime onset	Zygomaticus major: Negative < positive Corrugator supercilii: Negative > positive	 *d* = 0.32 *d* = 0.28	12
Ruiz-Padial et al.	2011	EMG startle (orbicularis oculi muscle regions): Difference in mV between peak and onset of the response	Unpleasant > neutral Unpleasant = pleasant	*d* = 0.32 *d* = 0.13[5]	11
Smith	1993	EMG activity: Mean of the last three readings during presentation	Negative = positive Negative = neutral	*d* = 0.04 *d* = 0.09	10
Sonnby-Borgström et al.	2003	EMG activity: Mean strength from stimulus onset to 2500 ms after onset	Zygomaticus major: Anger = happy Corrugator supercilii: Angry = happy	 *d* = 0.18 *d* = 0.09	9
Sonnby-Borgström et al.	2003	EMG activity: Mean strength from stimulus onset to 2500 ms after onset	Zygomaticus major: Anger = happy Corrugator supercilii: Angry = happy	 *d* = 0.18 *d* = 0.09	9

Note. *Abbreviations*: CS = Conditioned stimulus, US = Unconditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, s = Seconds, ms = Milliseconds, EMG = Electromyography, mV = Millivolts, n.r. = Not reported.

[1] Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, not always available due to missing information (e.g., cell sizes).

[2] Exposure durations: 25 ms, 80 ms, 250 ms, 500 ms, 1500 ms, 2000 ms, 5000 ms, and 6000 ms.

[3] Exposure durations: 25 ms, 40 ms, 50 ms, 80 ms, 150 ms, 250 ms, and 1000 ms.

[4] Results are reported for the control group only.

[5] Omnibus analysis of variance that includes the supraliminal condition. A significant interaction was found for exposure duration and stimulus valence, $F(2,54) = 7.69$, but the main effect of exposure duration was not significant.

Continues >

Cardiovascular (CV) activity. CV activity can be measured in different ways depending on the parameter of interest (for an overview see 95). Results are displayed in Table 7 and the operationalizations of CV activity are reported for each study. Notably, one fear conditioning study measured changes in HR acceleration and deceleration, but did not report on the outcomes (156). No other fear conditioning studies measured CV activity.

Priming studies. In 15 priming studies CV activity was measured and 58 contrasts were tested. Seven contrasts indicated that the negative affective stimuli resulted in a higher *systolic blood pressure* (SBP) compared to the control stimuli while five contrasts yielded no differences between the stimulus types. A higher *diastolic blood pressure (DBP)* to negative affective stimuli than to control stimuli was found in one of the ten contrasts. *Heart rate* (HR) for negative affective stimuli compared with control stimuli was higher in one contrast, but in 17 contrasts the HR was equal for both stimulus types. Five contrasts indicated a lower *pre-ejection period* (PEP) in response to negative affective stimuli compared to control stimuli, but three contrasts yielded no differences. The other CV parameters, *heart rate variability* (HRV), *total peripheral resistance* (TPR), *ventricular contractility* (VC), *cardiac output* (CO), *respiratory sinus arrhythmia* (RSA), and *respiratory rate* (RR) were only tested in a handful of studies. Two contrasts yielded no differences between stimulus types in HRV. One contrast indicated a higher, instead of the expected lower, RSA after negative affective stimuli and two other contrasts did not find a difference in RSA between stimulus types. One contrast found a higher TPR was found in response to negative affective stimuli compared to control stimuli and one other contrast found a lower CO. Finally, there were no differences between stimulus types on VC or RR.

Importantly, in two cases an increase of SBP reactivity was reported for sad faces, but not for angry faces, compared to the control stimuli. In four contrasts that yielded SBP increases the word 'angry' was used as a prime. In one contrast sad faces were used as a prime, which yielded higher SBP. However, another contrast did not indicate a difference between sad and control faces. Similarly, PEP responses to negative affective stimuli compared to control stimuli were found in response to sad and fearful faces, but not in response to angry faces, although one other contrast indicated that sad facial stimuli resulted in a higher PEP.

Conclusion and recommendations. Overall, 14 out of 58 contrasts (24%) indicated increased CV activity in response to subliminal negative affective stimuli compared with the control stimuli. The results suggest that some of the CV effects are related to the specific valence of the stimuli, resulting for example in different outcomes for 'angry' stimuli and 'sad' stimuli. However, the findings differed strongly across the

[6] Omnibus analysis of variance that includes the supraliminal condition. No significant interactions or main effects are reported.

various CV parameters. Still, the most consistent results emerged for SBP; it was larger in response to negative affective stimuli in seven out of 13 contrasts. In contrast, HR and DBP did not respond to specifically to negative affective stimuli. For PEP mixed results were apparent. Furthermore, for other parameters too few studies were found to summarize the findings. The ambiguity of the findings can be attributed to several methodological issues. Biobehavioral variables are often not reported on, such as recent eating, drinking, medication use and exercise prior to the study, while these factors influence the results greatly (189). Additionally, between-subjects comparison of impedance cardiography based outcome measures (e.g., CO, PEP, and TPR) is controversial; the absolute values are prone to various influences and the comparisons might not represent the state of the organism. To overcome this, within-subject designs are preferred (190). Here, this was only done by Garfinkel et al. (2016, 61); others studies used a between-subjects design. Notably, in this specific area of subliminal presentation CV measures are apparently rarely taken into account in fear conditioning studies.

In conclusion, with the exception of effects on SBP, little consistency is found in the literature concerning the effect of subliminally presented negative affective stimuli compared with control stimuli. These mixed findings are likely due to a wide variety of outcome measures based on different physiological features and measurement properties.

TABLE 7 Study outcomes for cardiovascular measures separately per manipulation type

Fear conditioning studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Peper and Karcher	2001	Accelerative HR changes: Max. HR 3 - 10 s after stimulus onset relative to the mean HR within a 10 s prestimulus interval. Deceleration HR changes: 0.5 - 3 s after stimulus onset	n.r.	-	10
Priming studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Chatelain and Gendolla (study 1)	2015	PEP, HR, SBP, and DBP: : Averages of last 4 min baseline subtracted from values during the priming and averaged over 1 min periods	PEP: Fear > happy Anger = happy HR: Fear = happy Anger = happy SBP: Fear = happy Anger = happy DBP: Fear = happy Anger = happy	 $d = 0.92$ $d = 0.33$ $d = 0.42$ $d = 0.06$ $d = 0.11$ $d = 0.32$ $d = 0.31$ $d = 0.20$	12
Garfinkel et al. (study 1)	2016	SBP, HR, and HRV: Baseline (2000 ms preceeding stimuli block) subtracted from averages of blocks of trials	SBP: Anger > relax HR: Anger = relax HRV: Anger = relax	$d = 0.57$ $d = 0.01$ $d = 0.17$	12
Garfinkel et al. (study 2)	2016	SBP, HR, and HRV: Baseline (2000 ms preceeding stimuli block) subtracted from averages of blocks of trials	SBP: Anger > relax HR: Anger = relax HRV: Anger = relax	$d = 0.61$ $d = 0.25$ $d = 0.41$	13
Gendolla and Silvestrini (study 1)	2011	PEP, SBP, DBP, and HR: Averages over 1 min periods. CV reactivity (average baseline values over last 4 min of habituation minus average values during the task)	PEP: Anger = happy Sad < happy SBP: Angry = happy Sad > happy DBP: Angry = happy Sad = happy HR: Angry = happy Sad = happy	 $d = 0.04$ $d = 0.04$ - - - - - -	13

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Gendolla and Silvestrini (study 2)	2011	PEP, SBP, DBP, and HR: Averages over 1 min periods. CV reactivity (average baseline values over last 4 min of habituation minus average values during the task)	PEP: Anger = happy Sad < happy SBP: Angry = happy Sad > happy DBP: Angry = happy Sad = happy HR: Angry = happy Sad = happy	 *d* = 0.07 - - - - - - -	13
Hull et al. (study 3)	2002	SBP, DBP, and HR: Average of two readings at each measurement period[2]	SBP: Angry > relax DBP: Angry > relax HR: Angry = relax	$\eta_p^2 = 0.12$ $\eta_p^2 = 0.15$ $\eta_p^2 = 0.08$	8
Hull et al. (study 4)	2002	SBP, DBP, and HR: Average of two readings within each measurement period[2]	SBP: Angry > relax DBP: Angry = relax HR: Angry > relax[3]	- - $\eta_p^2 = 0.08$	8
Jönsson and Sonnby-Borgström	2003	RSA: During 5 min in each condition. HR: Averages over 2 s before (baseline) and 7.5 s after onset of the stimulus	RSA: 17 ms: Angry > happy 56 ms: Angry = happy HR: 17 ms: Angry = happy 56 ms: Angry = happy	 *d* = 0.18 *d* = 0.04 *d* = 0.01 *d* = 0.04	10
Kemp-Wheeler and Hill	1987	HR and RR: Average of last 15 s prior to stimulus onset (baseline) compared with 15 s after stimulus offset	HR: Negative = neutral RR: Negative = neutral	- 	11
Lasauskaite et al.	2013	PEP: Averages of last 5 min baseline subtracted from task averages	Sad > happy	$\eta_p^2 = 0.17$	13
Lasauskaite Schüpbach et al.	2014	PEP, SBP, DBP, and HR: Averages of last 5 min baseline subtracted from task averages	PEP: Sad < happy[4] SBP: Sad = happy DBP: Sad = happy HR: Sad = happy	$\eta^2 = 0.03$ - - -	10
Lee and Tyrer	1981	HR, RR, and BP: N.r.	n.r.		11
Ravaja et al.	2004	RSA: Average of 45 s divided in 15-s epochs during primes. Natural logarithm was applied	Anger = happy Anger = neutral	*d* = 0.06 *d* = 0.04	14
Ruiz-Padial et al.	2011	HR: Task minus baseline in 0.5 s increments	Unpleasant = neutral Unpleasant = pleasant	- -	11

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Silvestrini and Gendolla	2011a	PEP, HR, SBP, DBP, and TPR: Averages of last 4 min of baseline subtracted from averages during the task	PEP: n.r. SBP: n.r. DBP: n.r. TPR: n.r.		11
Silvestrini and Gendolla	2011b	HR, SBP, and DBP: One min averages of last 2 min of baseline subtracted from those during the task	SBP: Sad > happy DBP: Sad = happy HR: Sad = happy	$d = 0.26$ - -	11
Weisbuch-Remington et al. (study 1)	2005	HR, VC, CO, and TPR: Average from last min of rest period subtracted from average value first min of the task	HR: Negative = positive VC: Negative = positive CO: Negative < positive TPR: Negative > positive	- - $d = 0.51$ $d = 0.51$	10
Weisbuch-Remington et al. (study 2)	2005	HR, VC, CO, and TPR: Average from last min of rest period subtracted from average value first min of the task	HR: n.r. VC: n.r. CO: n.r. TPR: n.r.	-	10

Note. *Abbreviations*: HR = Heart rate, s = Seconds, n.r. = Not reported, PEP = Pre-ejection period, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HRV = Heart rate variability, ms = Milliseconds, CV = Cardiovascular, RSA = Respiratory sinus arrhythmia (HF power), BP = Blood pressure, VC = Ventricular contractility, CO = Cardiac output, TPR = Total peripheral resistance.

[1] Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, not always available due to missing information (e.g., cell sizes).

[2] Baseline, after instructions, after practice trials, after the priming task, and during recovery.

[3] For HR $N = 32$.

[4] The study entailed a difficult and easy condition, but there were no differences between the conditions

Quality of the studies

The quality of the studies ranged from 5 to 14 out of a possible 15 points (Table 5 through 7) with a median quality rating of 10. Clearly, year of publication was of influence; most of the studies with a rating of nine or lower were published before 2000. This is understandable since science is not a static entity; some of the items are only recently considered to be vital, such as statements on conflict of interest or ethical approval. However, there are some exceptions. For example Flo et al. (2011, 137) scored a seven. The authors did not report on a blinding procedure of the researchers, blinded allocation to conditions, or an awareness check, means and standard deviations or other estimates of variation in the sample, and omitted results for some of the outcome measures. Overall, studies in this field could improve greatly by reporting effect sizes or providing the statistics and final cell sizes to be able to calculate the

effect sizes enabling the execution of meta-analyses (191). Additionally, the current results highlight the need of reporting sufficient detail about the study performed, the measures used, methods for data reduction, and agreement on these matters within the field. Together, these improvements would enable a more constructive scientific approach and accumulation of knowledge.

Discussion

With this systematic review we set-out to find all articles discussing empirical studies using subliminal negative affective stimuli while measuring the effect on CV, EDA, EMG, hormonal, and immunological activity in a healthy sample. We hypothesized that subliminally presented negative affective stimuli would increase peripheral physiological activity compared with a positive or neutral affective state. Overall, 60 (41%) of the 147 reported contrasts based on 65 studies revealed the expected effect, while four (2.7%) of the reported contrasts showed an opposite effect. The remaining portion (56%) did not find an effect. No studies were found that reported hormonal and immunological outcomes.

Within these mixed results some consistent findings were apparent. In fear conditioning studies, the expected effect was found relatively consistent for all EDA parameters while the other physiological outcomes have not been tested extensively. In the priming studies, the findings were mixed for the EDA parameters; there was a general absence of effects for SCR amplitude while some effects on SCR magnitude were apparent. Considering CV activity, a rather consistent effect on SBP was found but not on HR and DBP. No effects were found for EMG activity. However, little consistency in methodology was found across studies. There were major differences in stimulus types used (e.g., faces, words, with differences in valence), data handling, and outcome reporting. Furthermore, some outcome measures, such as PEP and TPR, have been understudied in this area. Not surprisingly, the included studies and outcomes have not previously been interpreted in terms of relevance to health. In sum, from the available literature it cannot be firmly concluded that subliminally presented negative affective stimuli affect peripheral physiological activity. The findings are inconsistent across the various physiological parameters and a low number of studies for each outcome measure has been performed. This warrants more and consistently conducted and reported research. These concerns will be addressed below.

The inconsistent results can be attributed to several factors. First, despite the use of comparable experimental methodologies (fear conditioning and priming, subliminal presentation of stimuli, and measurement of physiological parameters) the study designs and outcomes are framed within specific theoretical contexts. For example Gendolla and colleagues (e.g., 146,163,164) have focused on effort mobilization during a mental concentration task, which is expressed in PEP and is thought to be

modulated by subliminally presented faces that convey an emotion. Similarly, Öhman and colleagues have focused on the underlying mechanisms of fear from an evolutionary perspective by using fear conditioning to change EDA (e.g., 187). Furthermore, Hull and colleagues (e.g., 62) focused on the dynamics of self-regulation and effects on CV activity in after priming. These different specific theoretical frameworks result in subtle methodological differences that limit comparability of the results. Furthermore, only a few (partial) replication studies were found (e.g., 172, replicating 135; 61, partially replicating 62). This suggests that only a handful of cases attempted to replicate previous findings before trying a variation in the study designs. Again, this is likely due to the different perspectives between studies; they simply were not meant to provide systematic scientific progress. To sum-up, the inconsistencies found are at least partly the consequence of our attempt to gather 'field-independent' evidence.

Furthermore, the abundance of differences in stimulus presentation is likely to have influenced the overall findings. One salient example is the use of fear-relevant versus fear-irrelevant stimuli in the fear conditioning studies. Öhman (2009, 187) reasoned that conditioned fear-relevant images (e.g., of snakes) lead to stronger physiological responses compared to conditioned fear-irrelevant images (e.g., of mushrooms) suggesting an evolutionary advantage of being aware of snakes and spiders rather than of flowers or mushrooms. However, Lipp et al. (2014, 148) reported a fear conditioning study using snakes and wallabies (which are inherently cute and nonfear inducing, according to the authors) as stimuli and the same effect was found for both stimulus types. Moreover, Lapate, Rokers, Li, and Davidson (2014, 144) found a larger physiological response in their priming study to fearful faces compared to neutral faces, but not to spiders compared to neutral images. These differences add to the inconsistency in the findings. However, several other factors regarding stimulus presentation should also be considered. The studies by Codispoti et al. (2009, 133), Jönsson and Sonnby-Borgström (2003, 167), and Sonnby-Borgström et al. (2008, 168) indicate that *different presentation durations* might be of influence (i.e., participants might process stimuli presented for 50 ms or longer to a larger extent than shorter presentation durations). Moreover, it has been suggested that sensitivity to different presentation durations differs to a certain degree between individuals (46). Furthermore, the results from Wiens, Katkin, and Öhman (2003, 174) suggest an effect of *trial order* in conditioning (i.e., participants might learn the order of presentation and not the CS-US association when presentation of the CSs is not random). Additionally, using *words versus images* in affective priming may influence the findings, which is thought to depend on the specific task performed during priming (e.g., 192). Also, images of faces displaying *different emotions* appear to elicit specific physiological responses. Moreover, the physiological responses to sad faces seems to differ from responses to angry faces. This was also found by the second study of Chatelain and Gendolla (2015, 132), which was not included in this review

since it only used negative affective primes. Interestingly, a study by Verkuil et al. (2015, 193) measured sadness in a diary study and found that sadness interacted with gender in its effects on HRV over 24 hrs; while sadder women had a higher HRV, the opposite was true for men. This suggests that different emotional stimuli could elicit a different response between individuals (e.g., based on gender). A similar interaction was found by Jönsson and Sonnby-Börgström (2003, 141) in a study where gender was a predictor of the physiological response to angry faces. All in all, this suggests that used stimulus type and other design features can also affect the influence of negative affective stimuli presented outside of awareness on physiology.

Importantly, the type of awareness check used to test to what extend stimuli were successfully rendered subliminal also differed greatly. Since the advocacy by Merikle to more clearly define 'awareness' (1984, 121), it is considered appropriate to surpass subjective evaluations on awareness of the stimuli. Merely considering verbal report (e.g., asking 'could you see the image?' or 'do you hear something?') can be seen as an insufficient way to determine awareness of the stimuli. However, as Merikle and Daneman (2000, 194) indicated, it can be argued that objective measures can still be considered (partly) subjective as they are hindered by for example motivation and number of trials. Nowadays, the value of additional confidence ratings of awareness is recognized (122). Moreover, when considering the abundance of options and discussions on this matter, it is remarkable that several studies conducted after 1984 still only used verbal report. As a solution, Wiens and Öhman (2007, 51) advise to provide sufficient details about the checks performed (for a thorough discussion of this and related topics the reader is referred to 195-197). Overall, the methodological inconsistencies highlight the need for standardization of methods of subliminal priming and fear conditioning. Therefore, we strongly encourage the execution of systematic overviews of the literature in experimental psychology and debate on standardization of methodology.

To adequately interpret the findings of this systematic review some limitations should be kept in mind. First, we intentionally only included studies with a healthy sample to find the more general mechanisms that theoretically precede physical illnesses. Yet, research on the mechanisms of fear conditioning has also been performed with for example phobic individuals (for a review see 187). Since this group is thought be more vigilant to threatening information (198) the effects of the subliminal stimuli on physiological activity might be stronger compared to those of a healthy population. Thus, the restriction to healthy samples might have led to an underestimation of the effects.

Second, we included only studies that used stimuli that were negative affective for the general population, that is, not only negative affective for a specific subgroup of the general population. This excluded for example the studies performed by Levy, Hausdorff, Hencke, and Wei (2000, 199) who performed a subliminal priming study

with positive and negative stereotypes of ageing in a sample of 60 years and older. Another excluded study was done by Carlisle et al. (2012, 200) in which the participants were primed with personally determined social ties that varied in positivity and negativity. In both studies an effect of the negative affective stimulus on CV activity was found. Thus, it could be that specific samples respond to specific stimuli or that personalized negative affective stimuli may lead to a stronger physiological response, which would underestimate the results presented here.

Third, we included studies that presented subliminal stimuli, but the effects of supraliminal stimuli (presenting stimuli in such a way that subjects are usually aware of them), or both, were not evaluated. It might have been more adequate to test the effects of supraliminal as well as subliminal stimuli together, since in reality they are highly likely to co-occur (66). This was done by some studies (e.g., 133), but we did not further review these results. Future research should consider taking both methods of presentation into account to test whether subliminally presented negative affective information adds to the explanation of physiological activity in addition to conscious processes.

Fourth, we did not find studies with cortisol as an outcome measure, which may be surprising. However, in studies using subliminal presentation it would be impossible to adequately measure cortisol and relate this to the very subtle and fast manipulations as the change in glucocorticoids is relatively slow (e.g., 201) compared with for example changes in blood pressure. Thus, we have included glucocorticoids as an outcome measure, but the absence of findings is not surprising when taking into account the characteristics of the measure and this specific set of studies.

Finally, aside from one study, we did not include unpublished findings. This is a common problem for any review, but it might be particularly unfavorable in this case since the effects of interest were often of secondary importance in the reviewed studies. Therefore, it is reasonable to expect that when analysis of secondary importance were not statistically significant the results are even less likely to be published. To summarize the limitations, on the one hand the findings may underestimate effects because they only apply to a general healthy population and to generally stressful stimuli, but on the other hand the findings may overestimate effects when the problem of unpublished results is larger than anticipated.

The current study has two specific strengths. We introduce an elaborate procedure to find all relevant articles by producing an extensive keyword-profile to transcend field-dependent terminology. Usually, a rather typical keyword profile such as described as the initial profile in the Methods would be considered sufficient to find articles for a (systematic) review or meta-analysis. However, using an elaborated and systematically expanded profile, which includes evaluating the additional relevance of possible keywords from an exhaustive list, results in a far more comprehensive overview of the literature. In this case, out of the 54 articles 51 were found using the final

keyword-profile. In contrast, the initial keyword-profile had resulted in finding only nine of those articles. Furthermore, the three articles that we did not find with the keyword-profile either had no abstract or keywords and a nonspecific title or used author-specific terminology, and were found by checking the references of other included articles. Hence, without the elaborated keyword-profile we would have only reviewed 17% of the available literature on this topic. Evidently, regular keyword-profiles are insufficient and do not allow for a growing body of knowledge but rather a reinvention of the wheel within the limits provided by a theoretical paradigm. In sum, we strongly recommend using one final exactly formulated keyword profile that can be replicated by other researchers and used for later state-of-the-art-updates concerning the research question. Another strength of the current systematic review is the attempt to capture the quality of the studies. No standardized method to indicate the quality of experimental studies within the field of psychology has been previously incorporated in reviews. From the quality assessment it became apparent that studies often do not report essential information of the construct studied that is needed to estimate the effect or impact, replicate the findings, or perform meta-analyses. Thus, we advocate the implementation of a standardized quality assessment of psychological experimental research to stimulate adequate reporting of findings. Implementing a more comprehensive literature searching method combined with the exact key word formulation and standardized quality assessment would greatly enhance the impact and reliability of conclusions in the field.

To conclude, only part of the retrieved studies found an effect of subliminally presented negative affective stimuli on physiological parameters with the most convincing evidence emerging for SBP and SCR amplitude. However, the methodological differences and the insufficient number of studies for most parameters hinder firm statements about the effect. As research has demonstrated convincingly and consistently that information presented outside of awareness can affect brain activity and behavior, it seems pivotal to more systematically examine the possible contribution of processes outside of awareness on peripheral physiology to elucidate the relation between negative affect and health.

Chapter 3

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

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Abstract

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

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

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

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

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

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

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

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

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

Method

Participants

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

Instruments

Cardiovascular activity

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

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

Questionnaires

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

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

Subliminal priming task

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

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

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

TABLE 1 List of prime words used in the study

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

Note. The prime words were presented twice

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

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

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

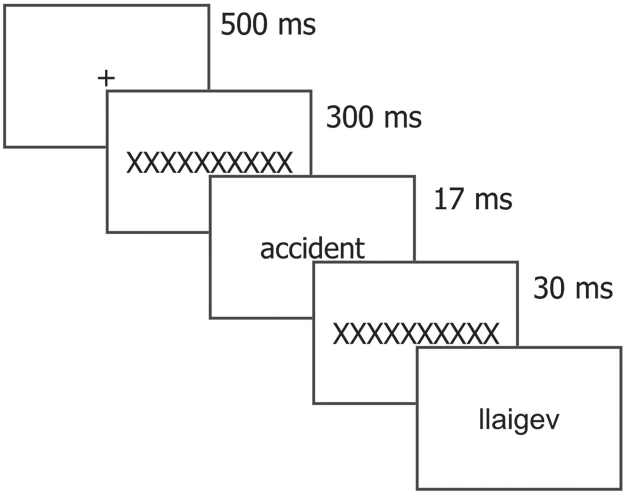

FIGURE 1 Depiction of the subliminal semantic priming procedure. Each trial started with a fixation cross (500 ms), followed by a forward mask (300 ms), a threatening or neutral prime (17 ms), a backward mask (30 ms), and a word/nonword as target that was presented until the participant responded or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

Procedure

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

Statistical analyses

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

Results

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

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

TABLE 2 Baseline characteristics stratified by condition

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

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

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

[+] $p < .10$

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

Valence and arousal words

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

Cardiovascular reactivity

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

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

Task performance

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

Moderating effects of trait worry and resting HRV

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

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

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

Exploratory analyses

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

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

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

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

Note. All predictor variables were centered and unstandardized regression coefficients are reported. Outcomes are expressed in change scores. MAP = Mean arterial pressure, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.
$^{+}p > .10$, $^{**}p > .01$, $^{***}p > .001$

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

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

Note. All predictor variables were centered and unstandardized regression coefficients are reported. Outcomes are expressed in change scores. MAP = Mean arterial pressure, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.
$^{+}p > .10$, $^{*}p > .05$, $^{**}p > .01$, $^{***}p > .001$

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

	Total (*N* = 54)		**Angry (*n* = 26)**		**Relax (*n* = 28)**		
Measure	*M*	*SD*	*M*	*SD*	*M*	*SD*	t/χ^2
Demographics							
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
Biobehavioral variables							
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
Cardiovascular measures							
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

	Angry (n = 26)		**Relax (n = 28)**			
Measure	*M*	*SD*	*M*	*SD*	*t*	*r*
Implicit affect						
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
Self-reported affect						
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 (ΔM = 1.06) and the recovery (ΔM = 0.99) when this participant was included. Results without this participant are reported to be conservative. For SBP, there was no significant Time×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×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×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 *p*s > .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

	Task				**Recovery**						
	Angry		*Relax*		*Angry*		*Relax*				
Outcome measure	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*[1]	*df*	
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×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 (*p*s > .10).

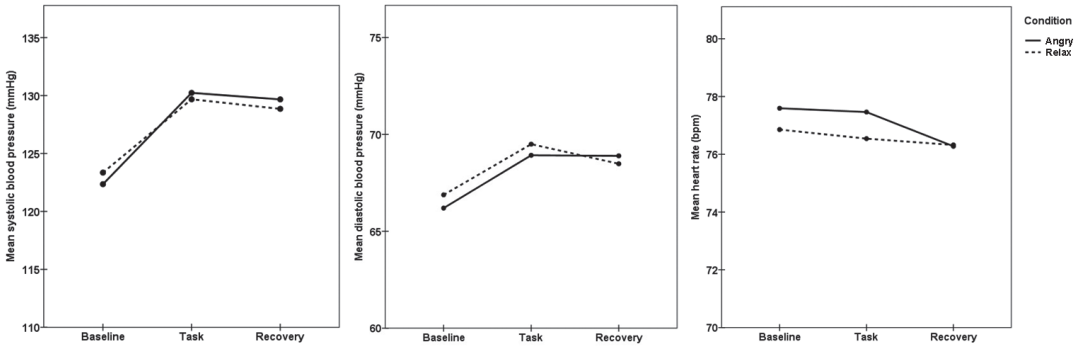

FIGURE 1 Cardiovascular activity throughout the experiment displayed per condition for systolic and diastolic blood pressure and heart rate

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 the 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.

Chapter 5

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

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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

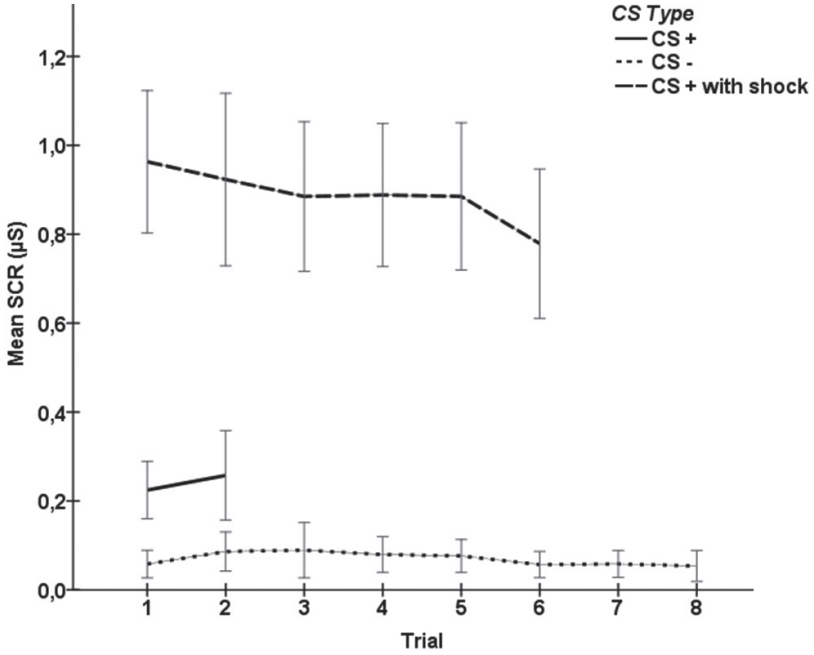


FIGURE 1 SCR magnitude (µS) in the acquisition phase for all CS types. Trial is presented as corresponding trial for each CS type and not in the actual order in which they were displayed in the experiment since stimuli were presented pseudo-randomly. The CS+ with US trials indicate the mean SCR magnitude 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.

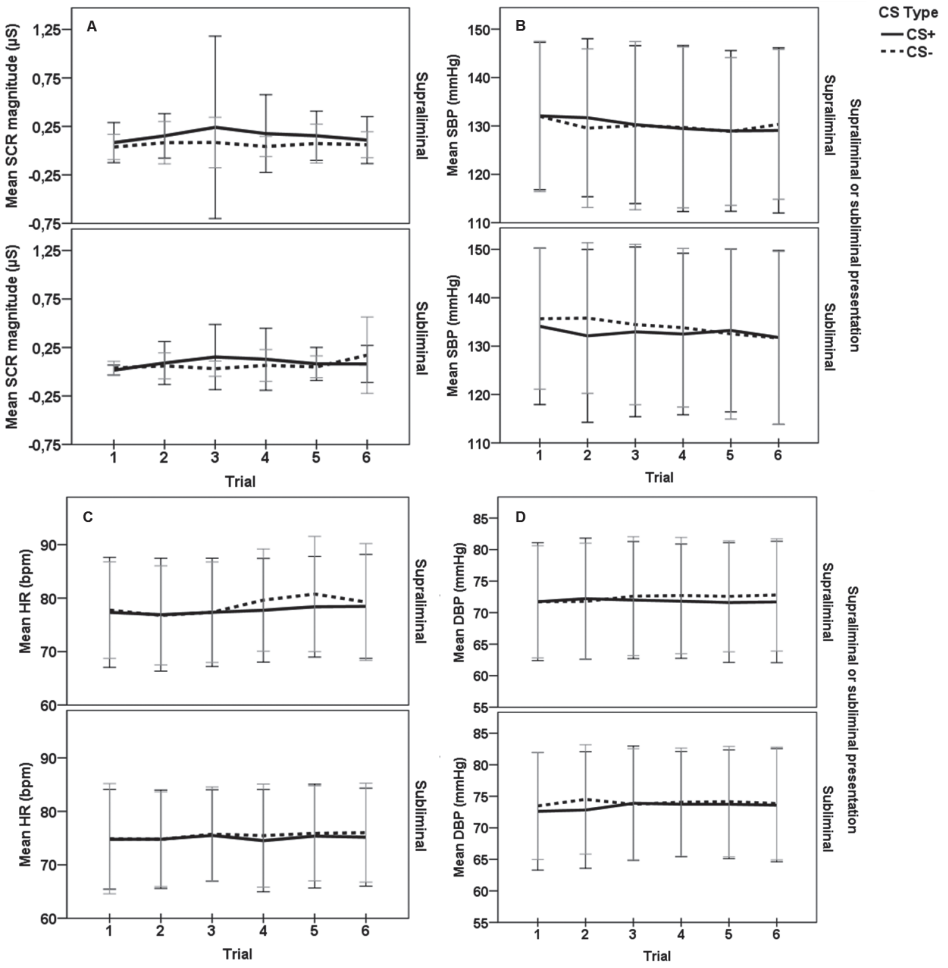


FIGURE 2 Test phase displayed for the CS+ and CS- presentation method for all outcome measures (A. SCR magnitude (µS), B. SBP (mmHg), C. HR (bpm), D. DBP (mmHg). The CS types were presented in two adjacent blocks of six trials, either subliminally or supraliminally, which are aggregated per trial number 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

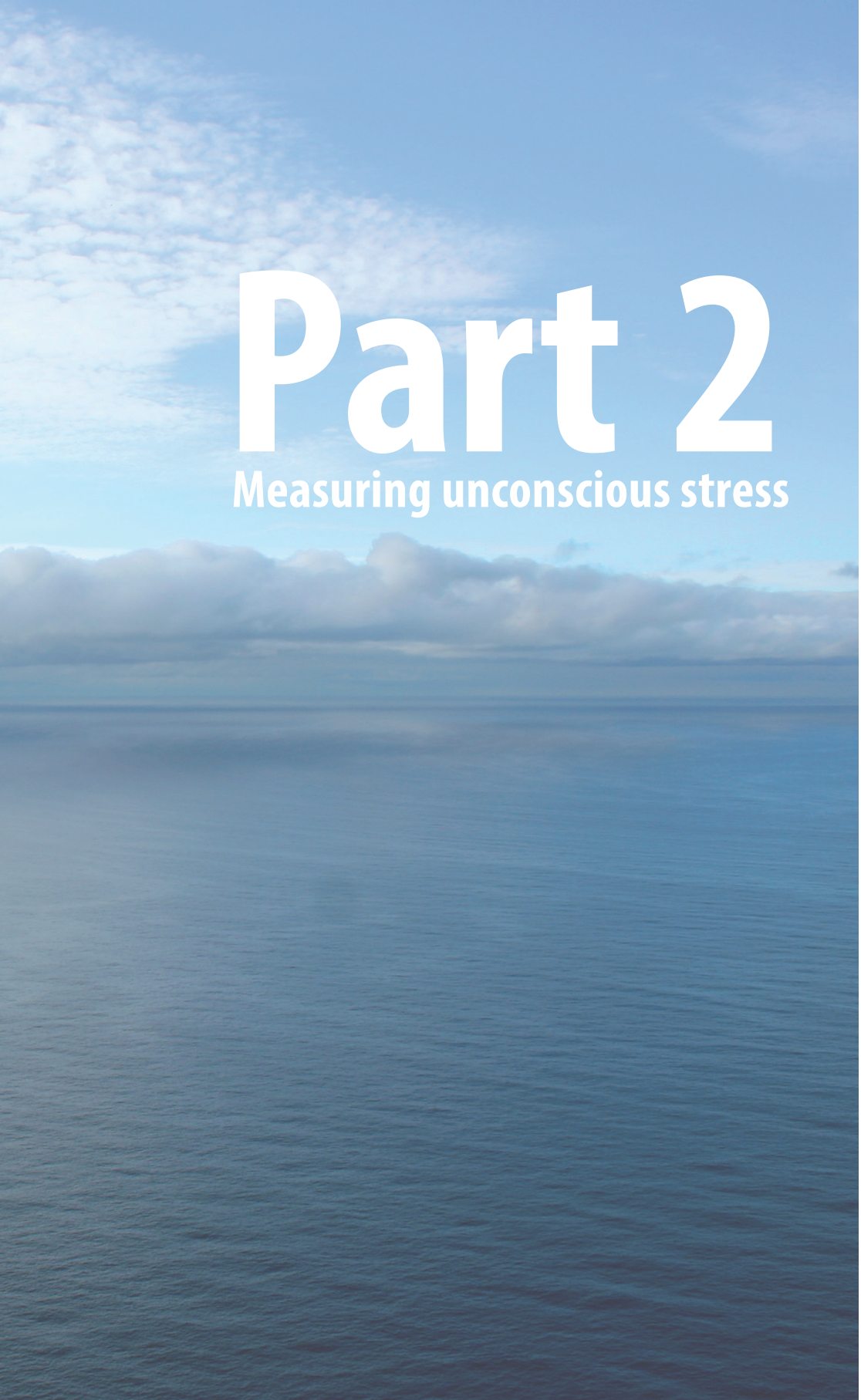
by HR acceleration. Furthermore, in a series of fear conditioning studies Castegnetti et al. (2016, 286) used HR period as index of fear memory in addition to SCR and found that overall the CS+ compared to the CS- elicited a small decrease at trial onset and a steep acceleration after 4.7 s following 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.



Part 2

Measuring unconscious stress

Chapter 6

The Implicit Positive And Negative Affect Test: Validity and relationship with cardiovascular stress-responses

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Abstract

Self-report (i.e., explicit) measures of affect cannot fully explain the cardiovascular (CV) responses to stressors. Measuring affect beyond self-report (i.e., using implicit measures) could add to our understanding of stress-related CV activity. The Implicit Positive and Negative Affect Test (IPANAT) was administered in two studies to test its ecological validity and relation with CV responses and self-report measures of affect. In study 1 students ($N = 34$) viewed four film clips inducing anger, happiness, fear, or no emotion, and completed the IPANAT and the Positive And Negative Affect Scale at baseline and after each clip. Implicit negative affect (INA) was higher and implicit positive affect (IPA) was lower after the anger inducing clip and vice versa after the happiness inducing clip. In study 2 students performed a stressful math task with ($n = 14$) or without anger harassment ($n = 15$) and completed the IPANAT and a Visual Analogue Scale as an explicit measure afterwards. Systolic (SBP), diastolic (DBP) blood pressure, heart rate (HR), heart rate variability (HRV) and total peripheral resistance (TPR) were recorded throughout. SBP and DBP were higher and TPR was lower in the harassment condition during the task with a prolonged effect on SBP and DBP during recovery. As expected, explicit negative affect (ENA) was higher and explicit positive affect (EPA) lower after harassment, but ENA and EPA were not related to CV activity. Although neither INA nor IPA differed between the tasks, during both tasks higher INA was related to higher SBP, lower HRV and lower TPR and to slower recovery of DBP after both tasks. Low IPA was related to slower recovery of SBP and DBP after the tasks. Implicit affect was not related to recovery of HR, HRV, and TPR. In conclusion, the IPANAT seems to respond to film clip-induced negative and positive affect and was related to CV activity during and after stressful tasks. These findings support the theory that implicitly measured affect can add to the explanation of prolonged stress-related CV responses that influence CV health.

Psychosocial stressors such as marital stress and job stress are increasingly recognized as contributors to the development or progress of cardiovascular (CV) disease (see for example 3,5,6,9,115,263-266). Still, studies have been inconclusive on the mechanisms underlying the relationship between psychosocial stress and CV diseases (8,9). This might be related to the inability of the used measurements of psychological stress to explain CV activity (23-25). The current paper addresses this issue by validating a test that indirectly assesses affect and is expected to more closely relate to psychophysiological responses; the Implicit Positive and Negative Affect Task (IPANAT; 84,291).

The reactivity hypothesis of stress has been the main focus of the field and emphasizes the acute physiological responses during a stressor. However, accumulating literature suggests that prolonged stress responses and not, or to a lesser extent, the reactivity during stressors, determine the detrimental consequences for health. In other words, measuring the CV activity during stressors might not fully represent that part of the physiological stress response that explains the development of CV or other diseases. Slow recovery from stressors and anticipatory responses to them might be of equal or even greater importance (10-16). Moreover, this prolonged activity leads to a pathological state that is often described as *allostatic load* (115) and is the final biological pathway to organic disease. Earlier research focusing on reactivity to a stressor has overlooked these different forms of the maladaptive stress response (i.e., prolonged physiological activation). These forms of prolonged activation have been attributed to ongoing cognitive representation of the stressors, which is known as perseverative cognition. Perseverative cognition, often manifested as rumination or worry, has been associated with prolonged CV activity (17-22, 292).

The assessment of psychological stress to explain related CV responses is typically done through self-report methods such as keeping a worry and mood diary or completing questionnaires like work stress scales or trait questionnaires of worry, anxiety, or general negative affect (e.g., 22-25,292,293). However, several findings indicate that these measures do not fully explain the prolonged CV responses to stressors (23-25). Brosschot et al. (2007, 22) for example found that individuals that experienced stressors and worry during the day displayed increased cardiac activity during sleeping at night, when conscious worry and affect-related cognitions are absent. Moreover, Pieper et al. (2010, 25) demonstrated that cardiac effects of worry in real life continued after worry episodes ceased and were not due to negative affect or biobehavioral variables such as movement or smoking. Additionally, Gerin and colleagues (23,24) found that slow blood pressure (BP) recovery after an experimental stressor was not due to explicit worrisome thoughts. These findings seem to indicate that part of the psychological stress response affects the CV system in a way that is not addressed by self-report measures. Brosschot and colleagues (26,27) have hypothesized that this part is explained by ongoing unconscious (or implicit) stress-related cognition. This unconscious stress-related cognition would represent a general negative state that

one is unable to express, but that does affect physical wellbeing. Concepts related to unconscious stress-related cognition have already been widely used within cognitive and social psychology, such as implicit affective attitudes, self-esteem, and emotion (see for example 39,43,44,53), and have been demonstrated to influence for example decision making processes (42) and affective evaluation (47). Implicit stress-related cognition cannot be measured with self-report methods, because for these methods deliberate processing of the assessed construct is required (74).

Various instruments have been designed to measure affective processing at an implicit level (i.e., implicit measures) such as the affective Implicit Association Test (IAT; 76,82) and the IPANAT (84). In the current study, we examined the IPANAT as an implicit measure of stress-related cognition operationalized as implicit affect (84). The IPANAT is suggested to operate as an implicit measure of affect through the process of affect misattribution (47,83,86,294). Similar to the original studies of Zajonc and colleagues (1980, 47) in the IPANAT ambiguous stimuli are presented, namely a set of nonsense words, of which the affective value is rated on a six point scale for 12 emotional adjectives. The assumption is that the participants, again as in Zajonc's studies, respond in accordance with their current affective state, without being fully aware of the construct being measured (84). The implict negative affect scale (INA) of the IPANAT has been shown to predict cortisol responses to a speech stressor and increases in circadian cortisol concentrations (85). The latter was recently partly replicated by Mossink, Verkuil, Burger, Tollenaar, and Brosschot (2015, 87). In Brosschot et al. (2014, study 2, 88) INA, measured with the IPANAT, was related to slower recovery of BP after a math stressor with anger harassment, whereas explicit negative affect (ENA) showed no significant relationship. However, in that study no control group for extra negative affective changes due to harassment was used, which limits inferences on the application of the IPANAT as implicit measure of stress-related cognition. In the current study, the harassment manipulation was again tested and a control group with only a math task was added to the design to test whether it is the specific affective component of anger harassment that affects INA and IPA as measured with the IPANAT.

The present studies address two issues. First, the IPANAT's content validity has hitherto only been tested with simple affective stimuli, namely pictorial emotional stimuli. Furthermore, although associations of the IPANAT with physiological measures have been found its relationship with explicit measures of affect are underappreciated (for a review see 83,84). For example Quirin, Kazen, Rohrmann, and Kuhl (2009, 85) found a relationship between the negative, but not the positive, subscales of implicit and explicit affect. However, this observational study measured changes in cortisol levels, but not in affect. Thus, the interpretation of both the relationship between implicit and explicit affect and the ability of the IPANAT to capture direct changes in affect due to stressful experiences cannot readily be applied to the current ideas

about unconscious stress-related cognition. In the current two studies content validity was examined under more realistic conditions by providing negative and positive emotional film clips in one study, which are more ecologically valid than simple pictures and have been suggested to elicit prolonged affective responses compared with pictures (e.g., 295-297), and by deploying a more naturalistic stressor, namely a math task with and without anger harassment in a second study. Moreover, in the first study we assessed the IPANAT's ability to detect changes in (implicit) affect and in the second study we relate the IPANAT subscales to physiological parameters to more specifically address the theory that changes in these parameters can be related to affect measured implicitly. We expected that the emotional film clips and especially anger harassment would evoke affect-congruent changes on the IPANAT subscales that are at least partly independent of explicit affect. Second, it addresses whether CV responses during a stressor and recovery from it, as a model of prolonged CV activation, are associated with implicit affect as measured with the IPANAT and whether this association is at least partly independent of that of explicit affect. More precisely, we expected that INA would be related to a higher reactivity to a stressor and slower recovery from it, and vice versa for implicit positive affect (IPA).

Furthermore, we expected stronger affective and CV effects for the math stressor with harassment. CV recovery is typically longer after emotional stressors than after physical or neutral stressors, while reactivity (i.e., responses during these stressors) is often equally high (e.g., 13,88 study 1). This difference in recovery is taken to be due to prolonged explicit stress-related cognition, or high ENA or low explicit positive affect (EPA), or both. Here, we hypothesized that it is also due to implicitly measured affect, that is, high INA or low IPA or both. Consequently, we expected that a more strongly negative emotional stressor (math with harassment) would lead to slower CV recovery and higher negative and lower positive affect, measured explicitly and implicitly, than a relatively more neutral stressor (math without harassment). We also expected that the slower CV recovery after harassment would be explained by the stronger affective responses, and that implicit affect explains CV recovery over and above explicit affect.

In sum, previous findings suggest that the IPANAT might be a suitable implicit measure of stress-related affective cognition, but its content validity and its ability to explain CV activity, expressed as reactivity and recovery to an emotional stressor, have not been thoroughly examined. In the present article two studies are reported that tested whether the IPANAT is able to detect changes in affective state induced by emotional film clips (study 1) and whether it can explain CV responses to a stressor beyond explicit measures of affect (study 2). In addition, it was tested whether the IPANAT scores were related to the general and differential CV responses to a stressor with and without anger harassment and to CV recovery after these stressors.

Study 1

Method

Participants and procedure

A total of 34 (64.7% female; mean age of 24.0 (*SD* = 8.51) students of Leiden University with sufficient understanding of the Dutch language enrolled in the experiment for course credits or five euro. Participants provided informed consent and received the standard instructions for the questionnaires after which they were seated in front of a computer and were asked to put on a Sennheiser HD201 headphone. In random order, four film clips were shown that were previously validated to elicit anger, happiness, fear, and a neutral state. The film clips were English versions identical to code 15 (1:17 min), 24 (2:45 min), 65 (3:57 min) and 55 (0:40 min), respectively, from the FilmStim database (297). The volume accompanying the film fragments was set at medium (45-55 dB). The IPANAT and Positive And Negative Affect Scale (PANAS; 251) were administered at baseline and after each video clip (see Figure 1). In one case the PANAS was not completed after the anger film clip. The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University, under number 5148415681.

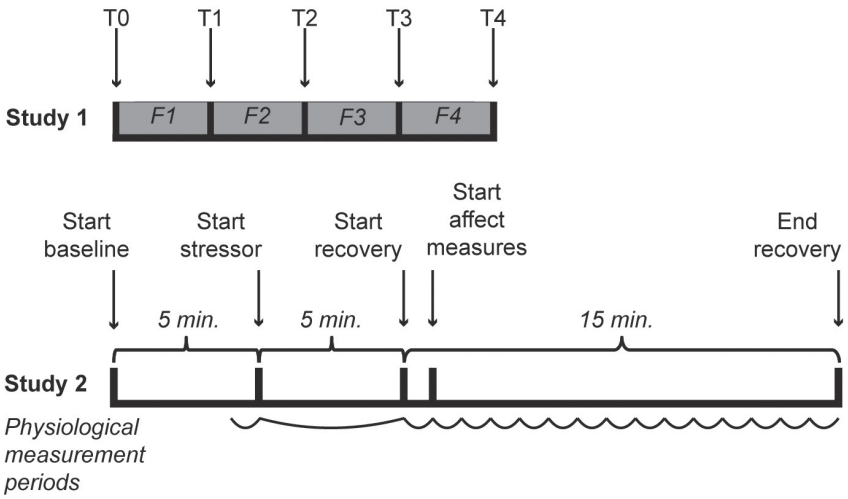

FIGURE 1 Timelines of both studies. In study 1 T0 represents the baselines measurement of affect, while T1 – T4 represent the affect measures after each film clip (indicated with F1 – F4). During study 2 cardiovascular activity was measured throughout. For analyses the last min of baseline, the five-min stressor and 15 separate min of the recovery were used, as indicated with a curved line

Implicit and explicit affect

A Dutch translation of the IPANAT as a measure of implicit affect was provided (84,88). Respondents rated six artificial words (vikes, tunba, ronpe, belni, sukov, safme) for emotional adjectives on a six-point Likert scale. In the version we used, the IPANAT for discrete emotions (83), 12 emotional adjectives are used. The mean scores per adjective for all artificial words were computed and summarized in the mean scores of INA (sad, gloomy, unhappy, annoyed, irritated, angry, afraid, frightened, scared) and IPA (joyful, cheerful, happy). In this particular study the IPANAT was used as a repeated measure by providing the entire IPANAT at baseline and two nonsense words, randomly selected from the pool of six words, after each film clip. Repeated presentation of the same full test was likely to cause carryover and training effects or boredom, resulting in erroneous scoring. Filling out the full version IPANAT takes about 5 min and as a repeated measure about 2 min for each administration. In the current sample the IPANAT administered at baseline was found to be reliable with Cronbach's α =.75 for INA and Cronbach's α =.89 for IPA, which is comparable to the reliability found by Quirin, Kazen and Kuhl (2009, 85).

At all measurement points explicit affect was measured with the PANAS, which measures positive and negative affect on two 10 item scales with emotional adjectives (251). Participants indicate on a five-point scale the extent to which the items apply to their current affective state. The PANAS was found reliable in this sample with Cronbach's α =.90 for ENA, Cronbach's α =.87 for explicit positive affect (EPA), which is comparable with reliability found by Crawford and Henry (2004, 298) in a large nonclinical sample. The implicit and explicit affective responses to video clips were compared with the affective responses at baseline.

Results

The demographical information of all participants is provided in Table 1. Mean affect scores are displayed in Table 2. In this within-subject design, the effect of the film clips on affect was determined with four one-way repeated measures ANOVA's, one for each affect measure. There were significant differences between film clips on all affect measures, INA: Wilks' $\lambda = .51$, $F(4, 30) = 7.32$, multivariate partial $\eta^2 = .49$; IPA: Wilks' $\lambda = .44$, $F(4, 30) = 9.64$, multivariate partial $\eta^2 = .56$; ENA: Wilks' $\lambda = .28$, $F(4, 29) = 18.6$, multivariate partial $\eta^2 = .72$; EPA: Wilks' $\lambda = .47$, $F(4, 29) = 8.31$, multivariate partial $\eta^2 = .53$, all $p < .001$.

Subsequently, affect after each film clip was compared with baseline through planned comparisons, tested one-sided since our hypotheses had a clear direction (e.g257). The results were corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (275-277). Results, displayed in Table 3, indicated that compared with baseline ($M = 2.55$, $SD = 0.53$) INA scores

were significantly higher after the anger inducing film clip (M = 3.00, SD = 1.01) and lower after the happiness inducing clip (M = 2.14, SD = 0.77), $t(33) = 2.79$, $p = .009$, $d = 0.56$ and $t(33) = -3.22$, $p = .003$, $d = 0.62$, respectively. INA was not significantly different after the fear inducing (M = 2.79, SD = 0.80) and neutral film (M = 2.59, SD = 0.81) clips compared with baseline, $t(33) = 1.59$, $p = .122$, $d = 0.35$ and $t(33) = 0.22$, $p = .830$, $d = 0.06$, respectively. Similarly, compared with baseline (M = 3.20, SD = 0.88), IPA was significantly lower after the anger inducing clip (M = 2.51, SD = 1.20), $t(33) = -2.83$, $p = .008$, $d = 0.66$ and significantly lower after the fear inducing clip (M = 2.67, SD = 0.84), $t(33) = -2.60$, $p = .014$, $d = 0.62$. IPA was significantly higher after the

TABLE 1 Baseline characteristics of the total sample (N = 34) of study 1

Measure	*M*	*SD*
Demographics		
Age, years	24.0	8.51
Female sex[a]	23	(70)
BMI	21.5	4.73
In a relationship[a]	19	(56)
Biobehavioural variables		
Smoke[a]	4	(12)
Smoked units today	0.08	0.28
Cafeine use[a]	29	(85)
Caffeine units today	0.45	1.03
Alcohol use[a]	12	(86)
Alcohol units last 24 h	0.39	1.77
Drug use[a]	4	(12)
Drugs today[a]	0	(0)
Current mental health complaints	2	(6)
Current psychological treatment	3	(9)

Abbreviations: BMI = Body mass index.

[a] Indicated with number of positive responses (percentage)

TABLE 2 Mean affect scores at baseline and after every film fragment in study 1

	Implicit				**Explicit**			
	NA		PA		NA		PA	
Phase	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Baseline	2.55	0.53	3.20	0.88	1.48	0.67	2.88	0.54
Anger	3.00	1.01	2.51	1.20	2.52[a]	0.90	2.38[a]	0.50
Happy	2.14	0.77	3.70	1.06	1.44	0.59	2.75	0.67
Fear	2.79	0.80	2.67	0.84	2.37	0.75	2.52	0.45
Neutral	2.59	0.81	2.95	1.06	1.45	0.54	2.30	0.61

Note. N = 34. *Abbreviations*: NA = Negative affect, PA = Positive affect.

[a] N = 33

happiness inducing film clip (M = 3.70, SD = 1.06), $t(33)$ = 2.46, p = .019, d = 0.51. IPA was not significantly changed after the neutral film clip (M = 2.95, SD = 1.06), $t(33)$ = 1.12, p = .272, d = 0.26.

ENA scores were, compared with baseline (M = 1.48, SD = 0.67), significantly higher after the anger inducing clip (M = 2.52, SD = 0.90) and the fear inducing clip (M = 2.37, SD = 0.75), $t(32)$ = 5.90, p < .001, d = 1.31 and $t(33)$ = 5.96, p < .001, d = 1.25, respectively. ENA was not significantly changed after the happiness inducing (M = 1.44, SD = 0.59) and neutral film clips (M = 1.45, SD = 0.54), $t(33)$ = -0.30, p = .767, d = 0.06 and $t(33)$ = -0.29, p = .772, d = 0.04, respectively. Finally, compared with baseline (M = 2.88, SD = 0.54), EPA was significantly lower after the anger inducing film clip (M = 2.38, SD = 0.50), the fear inducing film clip (M = 2.52, SD = 0.45) and the neutral film clip (M = 2.30, SD = 0.61), $t(32)$ = -4.92, p < .001, d = 0.96, $t(33)$ = -4.00, p < .001, d = 0.72 and $t(33)$ = -4.87, p < .001, d = 1.01, respectively. EPA was not significantly changed after

TABLE 3 Planned comparisons between affect at baseline and after each film clip in study 1

Comparisons	*M diff*	*SE*	*t*	*d*
Implicit NA				
Anger	0.453	0.16	2.79**	.56
Happy	-0.407	0.13	-3.22**	.62
Fear	0.245	0.15	1.59	.35
Neutral	0.033	0.15	0.22	.06
Implicit PA				
Anger	-0.691	0.24	-2.83**	.66
Happy	0.495	0.20	2.46*	.51
Fear	-0.534	0.21	-2.60*	.62
Neutral	-0.255	0.23	-1.12	.26
Explicit NA				
Anger[a]	1.027	0.17	5.90***	1.31
Happy	-0.032	0.11	-0.30	.06
Fear	0.891	0.15	5.96***	1.25
Neutral	-0.029	0.10	-0.29	.04
Explicit PA				
Anger[a]	-0.521	0.11	-4.92***	.96
Happy	-0.12	0.11	-1.19	.21
Fear	-0.359	0.09	-4.00***	.72
Neutral	-0.582	0.12	-4.87***	1.01

Note. N = 34. d is calculated with original means and standard deviations. Tests were performed one-sided and corrected for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995; Simes, 1986) with the false discovery rate set at 10%. *Abbreviations*: NA = Negative affect, PA = Positive affect.

[a] N = 33.

* p < 0.05, ** p < 0.01, *** p < 0.001

the happiness inducing film clip (M = 1.75, SD = 0.67), $t(33)$ = -1.19, p = .241, d = 0.21. Furthermore, there were no significant correlations between changes in implicit affect and explicit affect as displayed in Table 4.

TABLE 4 Pearsons product-moment correlations between changes in implicit and explicit affect in study 1

Affect	Fragment	*r*	
		ENA	EPA
INA	Anger	.26	.10
	Happy	.01	-.32^{+}
	Fear	-.07	.11
	Neutral	-.01	.33^{+}
IPA	Anger	-.06	.06
	Happy	-.06	.32^{+}
	Fear	.28	-.21
	Neutral	.10	-.34^{+}

Note. N = 34. *Abbreviations*: INA = Implicit negative affect, IPA = Implicit positive affect, ENA = Explicit negative affect, EPA = Explicit positive affect.
$^{+}$ $p < .10$

Discussion

In this study we tested whether the IPANAT is able to detect changes in affective state. The film clips instigated affect-congruent changes on the IPANAT subscales that were unrelated to changes in self-reported affect. These results add to the evidence for the IPANAT's validity by using stimuli that are more ecologically valid than the pictures used in the original studies (84). Notably, the fear inducing clip lowered IPA, but did not change INA, while the anger evoking clip did change both scales in the expected directions. The fear inducing clip might not have effectively evoked the targeted emotion, anxiety. Still, although not significantly, it did change INA in the expected direction, and yielded expected and significant explicit NA changes. Moreover, in the film clip pool (297) the same clip yielded a comparable mean ENA of 2.40. Together, this seems to indicate that the negative affect induced by the fear clip was not captured by the INA subscale of the IPANAT. Similarly, although explicit affect changed in an affect-congruent fashion, no changes in EPA were found after the happiness inducing clip. However, considering that EPA did not only decrease after the two negative clips, but also after the neutral film clip, the absence of an affect after the happiness inducing clip can be interpreted as an affect-congruent effect. An alternative explanation could be that the sample had a relatively high positive affect at baseline that did not change after the happiness inducing clip, as it was congruent

with the dominant affective state, but did decrease to a relatively more neutral state after the neutral film. Furthermore, one could argue that the differences in length of the film clips elicited different intensities of the induced affect (295). However, longer exposure time to a film clip did not increase the effect of the film clips (i.e., the fear inducing film clip was the longest but did not elicit the largest effect).

In sum, the results suggest that the IPANAT is able to measure changes in affect after emotion induction using films that are congruent with the valence of these stimuli. Moreover, it measures changes independently of explicit measures.

Study 2

Method

Participants

Thirty three Dutch undergraduate students from Leiden University, The Netherlands, were recruited and received eight euro or course credits for participation. Participants were randomly assigned to the stressor with harassment and stressor without harassment conditions (see below). Two participants had current CV disease and/or psychological problems, in one case the experiment failed due to technical difficulties and one participant had consumed over 5 units of alcohol in the 24 hrss before the experiment. These cases were excluded from the analysis. The final sample with a mean age of 21.0 (*SD* = 2.29) consisted of 18 females (62.1 %). The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University, under number 3145923676.

Implicit and explicit affect

The Dutch full version IPANAT was used in this study as a single measure one min after the termination of the stressor. The artificial word 'safme' was omitted as subjects reported it was associated with 'save me', and thus possibly not sufficiently ambiguous. Leaving out one of the words did not affect reliability; Cronbach's α was .93 for INA and .92 for IPA, which is in line with previous findings (84,88).

As an explicit measure of affect a Visual Analogue Scale (VAS) was provided. Participants were asked to what extent they felt a certain emotion (e.g., "How annoyed are you at this moment?"), using the same emotional adjectives as in the IPANAT. At the bottom of the screen a horizontal line of 10 cm was shown, with "not at all" on the left and "very much" on the right on which the participants could indicate their affect, resulting in a score in the range of -100 to +100, with a higher rating indicating increased levels of the adjective. Scores were averaged into ENA and EPA in a similar fashion as the IPANAT. With respect to reliability Cronbach's α's were .90 and .96 for ENA and EPA, respectively.

Cardiovascular activity

The physiological data were measured continuously throughout the experiment. Averages of each outcome measurement were calculated over the last min of baseline, the five-min stressor phase, and separately for all 15 min of the recovery. Systolic BP (SBP) and diastolic BP (DBP; mmHg) were measured with the Portapres Model-2 (Finapres Medical Systems, Amsterdam, The Netherlands), a noninvasive method to measure BP by placing a finger cuff on the middle finger of the nondominant hand. The electrocardiogram (ECG) was recorded with Kendall® 200 Covidien electrodes at a sample rate of 200 Hz with BIOPAC MP150 (Biopac Systems, Goleta, CA, USA) and visually inspected as well as corrected for movement artifacts with Acqknowledge 3.9.1.4. SBP, DBP, and HR (bpm) were extracted with a tailor made toolbox in Matlab R2012b. A low-pass filter (20 Hz, Blackman 40 coeffients) was applied to the BP signal. The ECG signal was up sampled to 1000 Hz and a comb filter (50 Hz, Q = 5) was applied. Root mean squared successive differences (RMSSD; ms) was derived from the ECG signal as a measure of HRV (95,299,301). Total peripheral resistance (TPR; mmHg.min/L) was derived using an approximation of cardiac output (CO) by the formula CO = (.002*(SBP – DBP))*HR (229,230,302). From MAP and the approximated CO, using the formula TPR = (MAP/CO), estimated TPR was then obtained (189). To avoid redundancy, only the outcome measure of interest, TPR, is reported.

Stress induction

All participants were instructed to perform a mathematical task; calculating backwards from 9000 in steps of 17 out loud. Emotional stress was induced by an anger harassment procedure in the stressor with harassment condition only; participants received seven pre-recorded remarks in an angry tone at set times (0:30; 1:00; 1:30; 2:30; 2:40; 4:00, and 4:55) during the five min duration of the stressor phase. These harassing remarks, such as "You are counting too slow, try to speed up." and "Could you really try to focus now?", were similar to those used by Radstaak, Geurts, Brosschot, Cillessen, and Kompier (2011, 303) and others (e.g., 304,305). Participants in the stressor without harassment condition did not receive any harassing remarks, but all participants received the instruction to start at 0:00.

Procedure

The study was run by two experimenters, of which one monitored the physiological measurements and the other was in contact with the participant. The procedure was explained to the participants after which they signed an informed consent before starting with the experiment. Demographics and biobehavioral variables were obtained followed by placement of the finger cuff and electrodes. The tasks and tests were presented via computer (E-Prime 2.0.8.90). A five min baseline period started during which participants could read a magazine with neutral content and were asked to sit

quietly (e.g., 92). This was followed by the stress induction as described above. The immediately ensuing recovery started with a min during which participants did not perform any tasks and were instructed to remain seated for measurement purposes. This was considered to be different from baseline since cognitive representations of the stressor were assumed to be present. After the first min of recovery the IPANAT started, followed by the VAS. When finished with the tasks within 15 min after the stressor, participants would wait until the 15 min had passed (See Figure 1). Finally, the finger cuff and electrodes were removed and participants were asked about their thoughts and experiences during and about the experiment before they were given a debriefing on the actual purpose of the study and constructs assessed with the IPANAT.

Statistical analyses

To represent reactivity, but not recovery, change scores were calculated by subtracting baseline values from those during the stressors for all CV outcomes (238) and effects of condition (i.e., stressor with and without harassment) were analyzed with one-sided *t* tests since our hypotheses had a specific direction (e.g., 257). Hierarchical multiple regression was used to assess the association between affect measures and physiological outcome variables, after controlling for condition. Recovery was analyzed with multilevel analyses for SBP and DBP (306), as it has various advantages over repeated measures ANOVAs when analyzing effects of time, such as a better handling of missing data and including individual slopes into the model, and thus is able to consider multiple levels in the data (e.g., 279). The mean of the CV measure during the stressors was included as covariate in the basic growth model. The model fit did not increase when adding both the baseline and task-related activity and by applying a random slope we already corrected for inter-individual variance unrelated to the stressor (278,279,306). Grand mean centering was applied to all predictors and covariates. For SBP and DBP separate models were built, but for all models Time was the level 1 variable, representing the measurements' course over 15 min (Model 1). Level 2 represented the person level, which included implicit (Model 2) or explicit affect (Model 3) or both (Model 4). The fit of the models was determined by significant changes in the Akaike information criterion (AIC) and Bayesian information criterion (BIC; 279). The data did not allow for multilevel analysis on HR, RMSSD, and TPR as visual inspection showed that recovery of these outcome measures occurred within one min after the stressor. Accordingly, for these outcome measures instead of multilevel analyses partial correlations were performed on the first min of the recovery phase with the affect measures while correcting for CV activity during the stressors. All analyses were done with SPSS 21.0.

Results

The data were inspected for collection errors, missing values, outliers (> 3 *SD*s from the mean), and violation of assumptions for all performed analyses. The distribution of RMSSD was skewed and a square root transformation was applied. One participant displayed a high SBP at rest (> 175 mmHg) and throughout the experiment, which was considered extreme. To be conservative, these data points were not included in analyses. Furthermore, one participant provided too many identical responses (i.e., 1-1-1-1 on the IPANAT) and the data were excluded from the data set. As suggested by Quintana and Heathers (2014, 307) differences between conditions regarding demographical and biobehavioral variables were examined but none were observed, nor were there differences found between conditions in CV outcome measures as displayed in Table 5.

Explicit and Implicit Affect

To examine the effect of the stressor with and without harassment on affect independent samples *t* tests were performed, one-sided (e.g., 257), and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (275-277). In response to the stressor higher levels of ENA were reported by participants after the stressor with harassment (M = -46.2, SD = 37.6) compared with the stressor without harassment (M = -74.97, SD = 21.89), $t(26) = 2.47$, $p = .020$, 95% *CI* [4.83, 52.6], $d = 0.93$. Furthermore, after the stressor with harassment lower EPA (M = -7.60, SD = 43.2) was reported compared with the stressor without harassment (M = 31.9, SD = 36.3), $t(27) = -2.67$, $p = .013$, 95% *CI* [-69.9, -9.15], $d = 0.99$. However, there was no condition effect on INA (with harassment: $M = 2.97$, $SD = 0.54$, without harassment: $M = 2.97$, $SD = 0.46$), $t(26) = 0.030$, $p = .976$, 95% *CI* [-0.38, 0.39], $d = 0.01$, nor on IPA (with harassment: $M = 3.34$, $SD = 0.75$, without harassment: $M = 3.43$, $SD = 0.52$), $t(26) = -0.37$, $p = .713$, 95% *CI* [-0.59, 0.41], $d = 0.14$. In sum, there was no condition effect on implicit affect, but there was an expected condition effect on ENA.

As exploratory analyses the associations between the affect measures were examined. INA was not significantly related to IPA or EPA, rs < -.20, ps > .05, or ENA, $r(28) = .16$, $p > .05$, IPA was not significantly related to ENA, $r(28) = -.20$, $p > .05$, and marginally significantly related to EPA, $r(28) = .32$, $p = .09$. ENA and EPA showed a strong inverse relationship, $r(28) = -.83$, $p < .001$.

Cardiovascular reactivity

First, we examined whether there were statistically significant changes in CV activity from baseline during both tasks using paired *t* tests, one-sided (e.g., 257), and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (275-277). Compared to baseline in both conditions there was an increase in SBP, DBP, and HR and a decrease in TPR (see Table 6). No significant

decrease was found for RMSSD. Second, we examined the effect of the stressor with and without harassment on the CV measures using independent samples *t* tests, again one-sided (e.g., 257) and with the Benjamini-Hochberg correction (275-277). These tests indicated that the stressor with harassment elicited significantly higher SBP (M = 23.3, SD = 9.43) compared with the stressor without harassment (M = 12.6, SD = 8.56), $t(25) = 3.07$, $p = .005$, 95% *CI* [3.51, 17.8], $d = 1.19$. DBP was significantly higher in the stressor with harassment (M = 12.9, SD = 1.40) compared with the stressor without harassment (M = 8.98, SD = 4.26), $t(26) = 2.27$, $p = .032$, 95% *CI* [-2.13, 0.09], $d = 1.61$,

TABLE 5 Baseline characteristics for the total sample of study 2 by condition

	Harassment (*n* = 14)		**No harassment (*n* = 15)**		
Measure	*M*	*SE*	*M*	*SE*	t/χ^2
Demographics					
Age, years	20.6	0.69	21.3	0.52	-0.73
Female sex [a]	7	(50)	11	(73)	1.68
BMI	21.7	0.91	22.2	1.07	-0.30
Biobehavioral variables					
Smoke[a]	2	(14)	1	(6)	-0.45
Daily Smoking	0.93	0.73	0.60	0.60	0.35
Cafeine use [a]	11	(79)	9	(60)	-1.17
Daily caffeine intake[c]	1.50	0.49	0.90	0.26	1.09
Alcohol use[a]	12	(86)	13	(87)	-0.01
Weekly alcohol consumption	3.09	0.76	2.72	0.97	0.30
Drug use[a]	1	(7)	0	(0)	-1.11
Exercise [a]	11	(79)	13	(87)	-0.33
Weekly exercise (hrss)	3.11	0.75	3.37	0.96	-0.21
Visits to GP (last 6 months)	0.79	0.21	1.00	0.45	-0.43
Cardiovascular measures					
SBP [b]	129.2	3.23	124.5	3.55	0.97
DBP	68.3	2.02	68.5	1.95	-0.16
HR	72.2	2.01	79.4	3.27	-1.93[+]
RMSSD [b]	6.14	0.41	5.78	0.35	0.66
TPR[b]	3.17	0.06	3.19	0.10	-0.16

Note. A square root transformation was applied to RMSSD. There were no significant differences between the conditions. *Abbreviations*: BMI = Body mass index, GP = General practitioner, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

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

[b] $N = 28$.

[c] Levene's Test indicated unequal variances, $df = 19.9$.

[+] $p < 0.10$, tested two-sided

respectively. Furthermore, TPR was significantly lower in the stressor with harassment condition (M = -1.44, SD = 0.42), compared with the stressor without harassment (M = -0.34, SD = 0.26), $t(18.62) = 3.07$, $p = .036$, 95% CI [-2.13, -0.08], $d = 1.16$, respectively. No significant differences ($p > .10$) in HR ($d = 0.62$) and RMSSD ($d = 0.12$) were found between conditions. These findings were confirmed by RM-ANOVAs. Gender, body mass index (BMI), and smoking were not related to the outcome measures and were not included in the models.

TABLE 6 Cardiovascular activity during manipulation in study 2

	Total Sample[a]			Condition				
				Harassment		*No Harassment*		
Measure[b]	*M*	*SE*	*t*	*M*	*SE*	*M*	*SE*	*t*
SBP	144.1	2.92	-8.75***	153.4	4.53	137.2	3.14	-3.07**
DBP	78.7	1.71	-11.6***	78.9	2.67	77.7	2.31	-2.27+
HR	85.2	1.89	-5.75***	82.8	3.36	86.8	2.51	-1.63
RMSSD	5.84	0.24	1.14	6.09	0.38	5.63	0.30	0.31
TPR	9.26	0.344	3.48**	8.67	0.497	9.74	0.478	2.33+

Note. All tests were performed one-sided and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10%. A square root transformation was applied to RMSSD. *Abbreviations*: SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

[a] Compared with baseline.

[b] Stressor with harassment has two missing values for SBP and RMSSD and one for DBP and HR. Stressor without harassment has one missing value RMSSD and TPR.

+ $p < 0.10$, ** $p < 0.01$, *** $p < 0.001$

Cardiovascular reactivity and affect

The association between implicit and explicit affect and CV reactivity was examined with a hierarchical regression analysis for each CV outcome measure resulting in five separate models. In all the models condition was added at step 1 and explicit affect at step 2. Since we expected that implicit affect would explain CV activity over and above explicit affect, we added INA and IPA in step 3. Even though ENA and EPA were highly correlated, $r(28) = -.83$, $p < .001$, VIF and tolerance were of acceptable levels in all tests and thus the assumption of multicollinearity was not violated (308). The final models are displayed in Table 7.

SBP was not significantly associated with ENA and EPA. However, INA and IPA were marginal significantly associated and explained an additional 16.1% of the variance, $F(5,19) = 2.60$, $p = .059$, $\Delta F = 2.58$, $p = .104$. The final model explained 40.7% of the variance, with condition, $t(24) = 2.10$, $p = .049$, and INA, $t(24) = 2.19$, $p = .041$,

TABLE 7 Summary of hierarchical multiple regressions for the CV change scores during the stressors in study 2

	SBP (mmHg)[a]			DBP (mmHg)[b]			HR (bpm)[b]			RMSSD (ms)[a,c]			TPR (mmHg.min/L)[d]		
	B	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*
Constant	-3.57	21.6		-3.57	9.78		-2.98	19.4		4.38*	1.82		3.33	1.98	
Condition	-10.0*	4.76	-.49	-3.11	2.28	-.34	-4.65	4.51	-.27	0.13	0.48	.07	-0.61	0.49	-.24
Explicit NA	-0.09	0.10	-.30	-0.08	0.05	-.56	0.07	0.09	.26	0.005	0.01	.20	-0.004	0.01	-.11
Explicit PA	-0.07	0.10	-.22	-0.05	0.05	-.49	0.03	0.09	.16	0.009	0.01	.39	0.01	0.01	.38
Implicit NA	8.54*	3.89	.40	2.10	1.86	.21	6.33	3.68	.34	-1.02*	0.38	-.52	-1.05*	0.40	-.41
Implicit PA	2.30	3.73	.14	2.83+	1.61	.38	1.40	3.18	.10	-0.42	0.35	-.27	-0.35	0.38	-.17
F		2.60+			1.98			1.35			1.79			5.27	
R^2		.41			.33			.25			.32			.34	
ΔR^2		.16			.15			.12			.30*			.20+	

Note. The table shows the associations between condition, affect and CV change scores as generated by the final model (step 3); condition was added at step 1, Explicit NA and PA at step 2, and Implicit NA and PA at step 3 to indicate the additional value of the implicit measure. The *F* statistic refers to that of the final model. ΔR^2 is the difference in explained variance between step 2 and step 3 the additional variance of the change scores explained by INA and IPA. *Abbreviations*: NA = Negative affect; PA = Positive affect, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

[a] $N = 25$.

[b] $N = 26$.

[c] A square root transformation was applied.

[d] $N = 24$.

+ $p < 0.10$, * $p < 0.05$

as significant univariate predictors. These results indicate that condition and a high level of INA were associated with an increased SBP. Regarding DBP, ENA, and EPA, nor INA and IPA were significantly associated with the outcome measure. However, in the final model IPA was a marginal significant univariate predictor, $t(25) = 1.76$, $p = .093$, (i.e., higher IPA, higher DBP). The total variance explained was 33.2%. HR reactivity was not associated with ENA and EPA, nor INA and IPA. Total variance explained, by condition, was 25.2%. For RMSSD, ENA, and EPA were not significantly associated. However, although INA and IPA did not significantly affect the model, $F(5,19) = 1.79$, $p = .16$, $\Delta R^2 = .30$, $\Delta F = 4.17$, $p = .032$, INA was a significant univariate predictor in the model, $t(24) = -2.67$, $p = .015$. The model explained 32.0% of the total variance and indicates that a higher INA was associated with a decrease in RMSSD during the stressor. Finally, reactivity of TPR was significantly associated with ENA and EPA at step 2 and explained 17.8% of the variance compared with step 1, $F(3,20) = 4.86$, $p = .011$, $\Delta R^2 = .18$, $\Delta F = 3.08$, $p = .07$. In the final model INA and IPA showed a significant association, $F(5,18) = 5.27$, $p = .004$, $\Delta R^2 = .172$, $\Delta F = 3.82$, $p = .041$, and explained 58.4% of the total variance. INA was the only significant univariate predictor in the model, $t(23) = -2.63$, $p = .017$. Again, a higher INA was related to a decrease in TPR during the stressor.

Cardiovascular recovery and affect

Multilevel modeling was applied to SBP and DBP. First, a growth model was fitted to the data to model the change over time, Model 1 (306). Second, two separate models for the implicit (Model 2) and explicit (Model 3) scales were fitted that included the affect scales and their interaction with Time and Time^2, to examine the relation of the affect measures independently. Finally, a model was fitted that included both subscales (Model 4), to examine the hypothesis that implicit affect can explain CV activity over and beyond explicit affect. The models were evaluated with and without condition as a predictor, but adding condition did not improve the models. Models without condition are reported.

To model SBP recovery, a heterogeneous autoregressive covariance structure was applied to the error variance, as is appropriate for fitting growth models (see for example 278). The slope of Time was allowed to vary randomly between participants. Results are displayed in Table 8a. There were significant associations of Time as well as Time^2, indicating that the recovery slope was composed of a linear decrease as well as a quadratic change (Model 1). The latter represented a trend with the fastest decrease at the beginning and a (small) increase in SBP towards the end of the recovery phase. Adding INA and IPA and their interactions with Time and Time^2 (Model 2) improved the model, $\Delta AIC = 70.8$ and $\Delta BIC = 48.1$. IPA in interaction with Time and Time^2 showed marginal significance, $B = -1.13$, $t(58.2) = -1.94$, $p = .057$ and $B = 0.06$, $t(43.6) = 1.90$, $p = .098$ respectively, indicating that higher IPA was related

to a stronger linear decrease of SBP and a stronger quadratic response. Thus, higher IPA was associated with a faster recovery of SBP, especially in the beginning of the recovery phase as displayed in Figure 2. By adding ENA and EPA (without implicit affect) and interactions with Time and Time^2 (Model 3), the fit also improved, Δ*AIC* =68.1 and Δ*BIC* = 45.5. However, no individual predictors were found. Additionally, the *AIC* and *BIC* were higher than Model 2, with -2.72 and -2.56 respectively, indicating a better fit of Model 3. When both implicit and explicit affect and interactions with Time and Time^2 were added to the model (Model 4), it was a better fit to the data compared with Model 1, Δ*AIC* = 141.1 and Δ*BIC* = 96.2, Model 2, Δ*AIC* = 70.3 and Δ*BIC* = 48.1, and Model 3, Δ*AIC* = 73.0 and Δ*BIC* = 50.7. The interactions of IPA and Time, *B* = -1.54, *t*(55.2) = 2.30, *p* = .025, and Time^2, *B* = 0.08, *t*(44.2) = 2.30, *p* = .026, were significantly associated with recovery of SBP in the final model. INA, ENA, and EPA were not associated with SBP.

To model DBP recovery, an autoregressive covariance structure was applied to the error variance, as is appropriate for fitting growth models (see for example 278). The slope of Time was allowed to vary randomly between participants. Results are displayed in Table 8b. There was a significant association of Time and Time^2, indicating that the recovery slope was composed of a linear increase as well as a quadratic change representing an increase at the beginning and an decrease in DBP towards the end of the recovery phase (Model 1). Adding INA and IPA and interactions with Time and Time^2 (Model 2) improved the model, Δ*AIC* = 80.0 and Δ*BIC* = 56.5. Here, INA showed a positive significant interaction with Time, *B* = 0.50, *t*(89.0) = 2.06, *p* = .043, and a negative significant interaction with Time^2, *B* = -0.04, *t*(67.4) = -2.26, *p* = .027. These associations indicate that higher INA was related to a smaller decrease in DBP with in fact a slight increase at first. Additionally, the IPA by Time interaction was

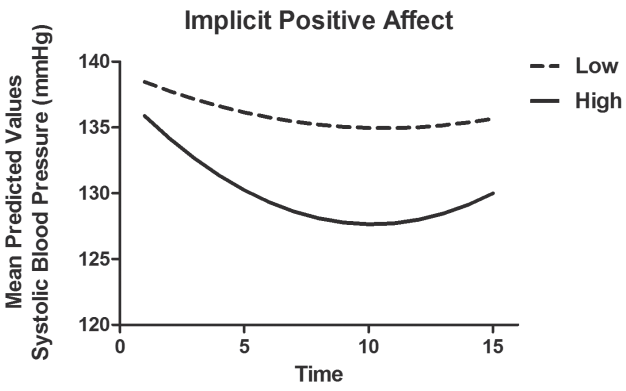

FIGURE 2 Mean predictive values of SBP over each of the 15 min of recovery (Model 4) displayed for high and low implicit positive affect. For display purposes scores of implicit positive affect were dichotomized

TABLE 8a Summary of multilevel analysis for recovery of SBP (mmHg)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	137.1	1.59	86.2***	137.0	1.60	85.6***	137.0	1.67	82.0***	136.8	1.70	80.4***
Time	-1.23	0.35	-3.54***	-1.34	0.34	-3.90***	-1.20	0.36	-3.37**	-1.35	0.35	-3.84***
Time^2	0.07	0.02	3.42**	0.07	0.02	3.81***	0.06	0.02	3.23**	0.07	0.02	3.70***
SBP Task	0.74	0.08	9.26***	0.75	0.09	8.61***	0.69	0.08	8.39***	0.73	0.09	7.76***
Implicit NA				-2.55	3.42	-0.75				-2.76	3.56	-0.78
Implicit PA				0.26	2.66	0.10				1.32	3.24	0.41
Time×Implicit NA				0.22	0.73	0.30				0.14	0.74	0.85
Time×Implicit PA				-1.13	0.58	-1.84+				-1.54	0.67	-2.30*
Time^2×Implicit NA				-0.03	0.04	-0.77				-0.03	0.04	-0.73
Time^2×Implicit PA				0.06	0.03	1.90+				0.08	0.03	2.30*
Explicit NA							-0.06	0.09	-0.70	-0.05	0.09	-0.53
Explicit PA							-0.07	0.07	-0.94	-0.06	0.08	-0.76
Time×Explicit NA							0.01	0.02	0.76	0.02	0.02	0.93
Time×Explicit PA							0.005	0.02	0.29	0.02	0.02	1.28
Time^2×Explicit NA							-0.0003	0.001	-0.26	-0.0008	0.001	-0.73
Time^2×Explicit PA							-0.0003	0.0009	-0.36	-0.001	0.0009	-1.22
AIC		2347.5			2276.7			2279.4			2206.4	
BIC		2438.8			2390.7			2393.3			2342.6	
N		23			29			29			35	

Note. Error at Level-1 was organized with a heterogeneous autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. *Abbreviations*: SBP = Systolic blood pressure, NA = Negative affect, PA = Positive affect, 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 8b Summary of multilevel analysis for recovery of DBP (mmHg)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	71.5	0.62	115.8***	71.3	0.56	126.0***	71.6	0.61	116.5***	71.3	0.58	122.8***
Time	0.25	0.12	2.46*	0.22	0.11	1.99*	0.29	0.11	2.55*	0.26	0.11	2.38*
Time^2	-0.02	0.008	-2.46**	-0.02	0.007	-2.30*	-0.02	0.007	-2.70**	-0.02	0.007	-2.60*
DBP Task	0.85	0.07	13.06***	0.94	0.07	14.1***	0.81	0.07	12.3***	0.91	0.07	12.5***
Implicit NA				0.69	1.24	-0.56				-0.76	1.27	-0.59
Implicit PA				-0.81	0.94	-0.86				-0.30	1.09	-0.28
Time×Implicit NA				0.50	0.24	2.06*				0.59	0.24	2.45*
Time×Implicit PA				-0.45	0.18	-2.46*				-0.41	0.19	-2.15*
Time^2×Implicit NA				-0.04	0.02	-2.26*				-0.04	0.02	-2.66**
Time^2×Implicit PA				0.01	0.01	1.21				0.02	0.01	1.22
Explicit NA							-0.04	0.03	-1.18	-0.02	0.03	-1.09
Explicit PA							-0.04	0.03	-0.04	-0.02	0.03	-0.85
Time×Explicit NA							-0.006	0.006	-0.93	-0.007	0.006	-1.09
Time×Explicit PA							-0.005	0.005	-1.12	-0.004	0.005	-0.75
Time^2×Explicit NA							0.0003	0.0004	0.73	0.0004	0.0004	1.11
Time^2×Explicit PA							0.0003	0.0003	0.41	0.001	0.0003	0.40
AIC		1732.7			1652.7			1669.6			1593.5	
BIC		1768.7			1712.3			1729.1			1676.0	
N		9			15			15			21	

Note. Error at Level-1 was organized with an autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. *Abbreviations*: DBP = Diastolic blood pressure, NA = Negative affect, PA = Positive affect, AIC = Akaike information criterion, BIC = Bayesian information criterion, *N* = Number of parameters.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

significant, B = -0.45, $t(89.5)$ = -2.46, p = .016, indicating that higher IPA was related to a faster linear recovery of DBP over time. Adding EPA and ENA to the model did not substantially improve the model (Model 3). When both implicit and explicit affect and interactions with Time and Time^2 were added to the model (Model 4), the fit did not improve and the associations between implicit affect and DBP recovery remained. The results are illustrated in Figure 3. Separate models of both SBP and DBP were also run with gender, BMI, and smoking as covariates. Adding these covariates to the models did not change the associations of implicit and explicit affect with SBP and DBP recovery.

As mentioned, before recovery of the other outcome measures took place within one min after the stressors had ended and could therefore not be modelled over time using multilevel analysis. Alternatively, to test the association with the affect measures partial correlations were performed on the first min after recovery of the means of HR, RMSSD, and TPR, correcting for the preceding reactivity. HR, RMSSD, and TPR were not significantly related to implicit or explicit affect. Results are displayed in Table 9.

TABLE 9 Pearson product-moment partial correlations between measures of affect and first min of recovery of study 2

Affect	HR[a]	RMSSD[b]	TPR[b]
Implicit NA	-.24	.30	.17
Implicit PA	-.20	-.14	.25
Explicit NA	-.18	-.001	.18
Explicit PA	.16	-.05	-23

Note. Controlled for HR, RMSSD, and TPR during the stressor. A square root transformation was applied to RMSSD. There were no significant correlations. *Abbreviations*: NA = Negative affect, PA = Positive affect, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

[a] N = 23

[b] N = 22

Discussion

Study 2 examined whether affect measured at an implicit level, as measured with the IPANAT, was associated with CV reactivity to and CV recovery after a stressor with or without anger harassment. During both stressors participants showed increased SBP, DBP, and HR, and lower TPR compared with baseline. When comparing the two conditions, these associations were more pronounced for SBP, DBP, and TPR after the stressor with harassment compared with the stressor without harassment. HR and RMSSD responses were similar for both conditions. Taken together this suggests a more pronounced cardiac controlled vascular response during harassment in addition to a math stressor.

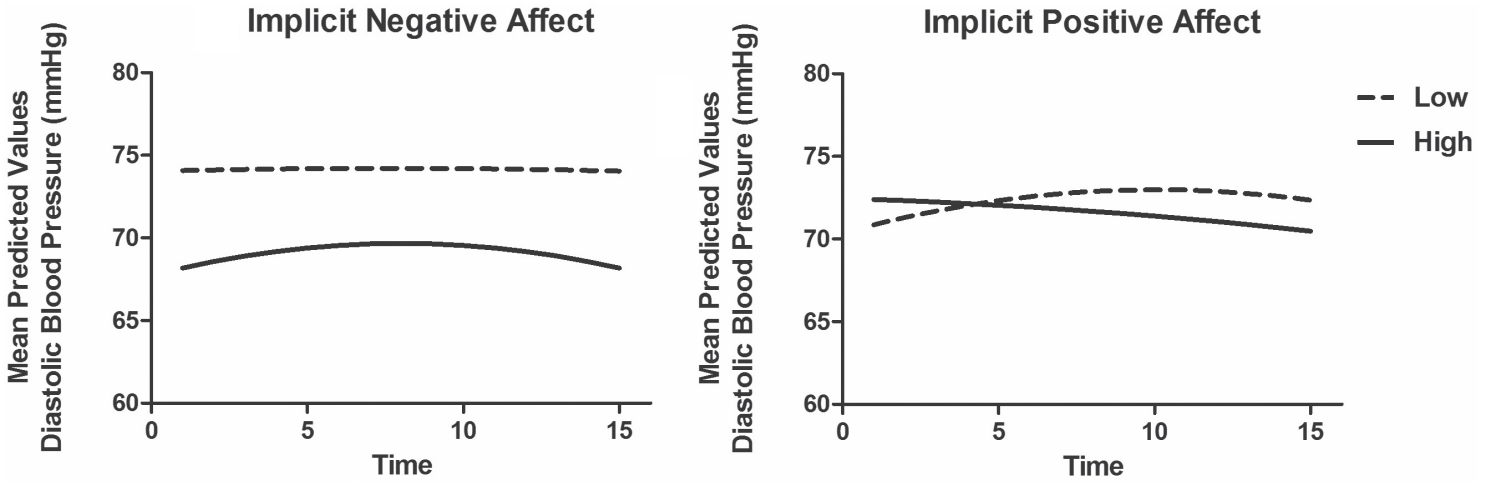

FIGURE 3 Mean predictive values of DBP over each of the 15 min of recovery (Model 4) displayed for high and low implicit negative affect and high and low implicit positive affect. For display purposes scores of implicit affect were dichotomized

There were no differences between the conditions in implicit affect. In contrast, those in the stressor with harassment condition experienced more ENA and less EPA as expected. This indicates that the more negative affective component of the harassment stressor was only reflected in explicit affect and not in implicit affect. However, higher INA was related to higher SBP reactivity and lower RMSSD and TPR reactivity *during* the stressors independent of stressor type. No associations between implicit affect and DBP and HR levels were observed during the stressors. Unexpectedly, the pattern of recovery was similar for both conditions. Overall, BP recovered rather slowly after an initial somewhat faster decrease. Importantly, the slow recovery of BP over the course of the recovery was (partly) statistically explained by implicit affect, but not by explicit affect. More precisely, slow recovery of SBP was related to low IPA, but not to INA. Slow recovery of DBP was partly related to both high INA and low IPA. HR, RMSSD, and TPR seem to have recovered rather quickly, that is, within the first min after the stressor. For these outcome measures no relationship with implicit affect measures was found. Remarkably, explicit affect was not related to any of the CV measures.

Taken together, the most salient result of study 2 seems to be that not explicit, but implicit affect explained variance in reactivity and recovery, but that at the same time explicit, but not implicit affect, was influenced by the stressor types, and thus by the experimental increase in negative emotionality. One explanation of these contrasting results might be that self-reported (explicit) affect reflected mainly the experimental demand characteristic ("the experimenter made me angry so I think I am angry") while implicit affect reflected the core affective state induced by both stressors (310), which was not substantially influenced by the harassment, as will be discussed below.

General discussion

Traditional self-report measurements of stress, or explicit measures of affect, cannot fully explain CV activity. Hence, the relationship between affect as an indicator of psychological stress and CV health remains largely indeterminate, and the examination of a possible role for implicit measures of affect is warranted. In the present work the IPANAT, as a promising implicit measure of affect, was evaluated in two studies to examine its ability to assess changes in affective state and explain stress-related CV activity beyond explicit measures of affect. In study 1 the IPANAT appeared to be able to measure affect-congruent changes in INA and IPA after anger and happiness inducing film clips. Of the multiple expected congruent effects only an effect on IPA, but not INA, after a fear inducing clip was found. Importantly, implicit affect changed independently from explicit affect. Thus, the IPANAT is able to measure changes in affect that are generally congruent with the valence of the presented stimuli and

independent of explicit affect. We conclude that the differential responses of the IPANAT in response to the film clips form an important extension of the modest number of available validation studies of the IPANAT and add ecological validity to previously used methods (e.g., pictorial stimuli).

Study 2 employed a realistic stressor with and without an enhanced negative affective component and continuously measured CV activity. The affective component was reflected in differences in explicit affect, but not in implicit affect. Nevertheless, only the implicit affect measures, and not the explicit ones, were associated with the CV responses to both stressors and their recovery afterwards. Specifically, SBP increases and HRV and TPR decreases during the stressors were related to higher INA, but implicit affect did not clearly relate to DBP and HR reactivity. Slower recovery of SBP was associated with lower levels of IPA, and DBP recovery was associated with both IPA and INA in the expected direction. HR, HRV, and TPR showed a very quick recovery that was not related to implicit or explicit affect. Thus, the IPANAT adds to the understanding of the CV response to stressors were explicit measure do not. These results and some unexpected findings, such as the prolonged physiological effects of the stressors on BP but not HR, HRV, or TPR, are discussed in more detail below.

Stressors and CV activity

We did not find a direct effect of the manipulation of the stressors on recovery, but the differences in recovery can be attributed to the differences in reactivity. The stressors yielded higher SBP and DBP and lower TPR, and for all CV measures the magnitude of reactivity contributed to speed of recovery. This suggests a role for the reactivity, not the stressor itself, in the effect of a stressor on the speed of CV recovery. Consequently, the notion of Brosschot et al. (2014, 88) and Linden et al. (1997, 13) that an emotional stressor would delay CV recovery compared with nonemotional stressor holds to the extent that it increases reactivity that, independent from condition, slows down recovery.

In general, the pattern of CV activity in study 2, a vascular (i.e., BP) and myocardial (i.e., HR) increase during the stressor and a prolonged recovery that appeared to be mostly vascular under cardiac control, is comparable to other studies (e.g., 8,10,19,304,305,309). The quick recovery of HR is in line with the observation that an increase in HR can be seen as primarily reflecting task engagement or effort (e.g., 311), and less related to possible emotional aspects of the task that might linger on after its completion. Furthermore, the speech activity required in the current stress task (i.e., calculating loudly) might also have played a role. Sloan, Korten, and Myers (1991, 312) found a smaller increase in HR during a mathematical task when vocalization of the response was not required. More specifically, changes in respiratory frequency due to speaking were found to increase HR. The neccesity to speak ended right after the task resulting in a quick decrease of HR. Sloan and colleagues (1991, 312) also

attributed the absence of changes in HRV to the effect of speaking on HRV. Thus, the findings regarding HR and HRV might not or to a lesser extent be related to the psychological component of the stressors but rather to the design characteristcs of the study.

In contrast to what is commonly found in threatening situations, namely an increase in TPR, we here found a decrease in TPR (99,100). It is possible that the stressors, a mathematical task with or without harassment, did not induce a threatened but a challenged state. Regarding our findings with TPR the stressors might not have been as straining as we had anticipated, for example because of lack of personal relevance of the stressors to the participants (99). The findings also suggests that the prolonged effects on SBP and DBP cannot be explained by TPR, that recovered within a min after the stressor, but are due to other factors that we have not measured directly, such as stroke volume or cardiac output. Overall, the results support previous notions that researchers should include recovery in the laboratory models of stress, as the activity seen during reactivity does not necessarily reflect clinically relevant responses (13).

IPANAT and CV activity

The findings of study 1 add to the understanding of affect, measured at an implicit and explicit level, by addressing the ongoing nature of affect through presentation of film clips. Furthermore, the absence of changes in INA after the fear inducing clip is similar to the study of Quirin, Bode, and Kuhl (2011, 253) in which they showed a threat-related film clip and measured INA and IPA but found no changes in implicit affect after a threat inducing film clip. This suggests that the INA subscale might not be sensitive or specific enough to detect fear. The construct validity, both convergent and discriminant, seem supported by study 1: the scores on the IPANAT scales are reasonably congruent with the emotional content of the different emotional film clips. This was only partially the case for study 2 where only convergent validity seems apparent from the expected correlations with physiological measurements stress responses. In line with previous research, we observed no association between INA and IPA, which explains why the results we found with INA did not always mirror those with IPA (84,85).

The stressors in study 2 led to group specific changes in explicit but not implicit affect. This is even more surprising considering the independent relation we found between implicit affect and CV outcome measures. The increased ENA and decreased EPA can be explained by demand characteristics of the stressors. In the condition with harassment the affective component was quite obvious to the participants. They were told they were not doing a good job. In the stressor group without harassment there was no feedback which created an ambiguous setting. These differences might very well be what was measured with the explicit measures of affect; the ambiguous situation was not experienced as overtly negative. An alternative explanation is that

in study 2 that the IPANAT scores were in fact related to the trait component, and not the state component, of affect (84). As no baseline measure of the IPANAT was taken, the current study does not exclude this possibility; perhaps it is the trait part of affect captured by the IPANAT that is related to CV activity. However, it is likely that self-reported affect reflected what the participants thought they had to report and not necessarily how they were feeling (i.e., their core affect; 310). Moreover, core affect might be best reflected on the IPANAT subscales; both stressors elicited discomfort which was overridden by demand characteristics of the experiment on the explicit level of affect but was displayed in both conditions on the implicit level. This explanation is further amplified by the finding that only implicitly measured affect contributed to CV activity during and after the stressors. If this interpretation is correct, implicit affect scores reflected core affect that was manifested in CV changes. This highlights the additional value of implicit measures, or the IPANAT in particular, in addressing the relation between stress and CV diseases (76,77,82).

The role of positive affect in the development of disease has not been explicitly addressed in the unconscious perseverative cognition hypothesis, which emphasizes the health consequences of stress-related cognition beyond awareness (e.g., 27). However, in the current study we found that a higher IPA is related to higher DBP reactivity and lower IPA is related to slow recovery of both SBP and DBP. This is consistent with the results of Quirin and colleagues (85, study 1) who found that increased IPA, not INA, measured during two days, was related to a lower cortisol awakening response and total diurnal cortisol the following day in addition to EPA. The finding that IPA is related to CV activity and cortisol excretion provides new insights in the relation between the IPANAT and two biological mechanisms.

Overall, the prolonged BP responses were best explained by implicit affect more than any other variable measured. Together these results suggest that stress-related cognition beyond self-report is related to physiological effects of stress, but, importantly, reduced levels of IPA play an equally detrimental role.

Limitations

The results should be interpreted while considering some limitations. In study 2 the sample sizes, particularly regarding the two conditions, were rather small which increases the risk for Type 2 error (i.e., the study may have been underpowered to reveal statistical significant findings). In this light we have interpreted marginal statistically significant findings in both studies as potentially relevant, which was supported by the effect sizes. Furthermore, in study 2 there was no neutral condition, merely a mathematical task with and without anger harassment. No differences between conditions were found for affect measured at an implicit level and CV recovery. Adding a true neutral condition without a stressor might provide additional information about the ability of the IPANAT to detect INA induced by a psychological stressor and enabling

inferences about the role of affect, measured implicitly and explicitly, in physiological recovery. Alternatively other methods of stress induction could be considered, such as a public speech stressor or the Trier Social Stress Test, which combines a public speech with the anger harassment used in study 2 (e.g., 313). Also, we cannot exclude the possibility that participants differed, despite randomization, in natural math-related abilities, which could have been a confounder. Finally, study 1 and 2 did not use the same explicit measures and can therefore not be readily compared; it cannot be excluded, for example, that we would have found associations of explicit affect with CV activity in study 2 if we had used the PANAS used in study 1. To further clarify the relation between implicit stress-related cognition and CV health, future studies should not be limited to implicit measures of affect after experimentally induced stress, but should also apply the measures to daily life (87) and/or in individuals with chronic stress. Finally, the current experiments focused on the assessment of implicit affect with the IPANAT. However, other measures of implicit constructs to assess other aspects of unconscious stress-related cognition (e.g., action tendencies or emotion recognition) could also provide more information to clarify the relation between psychological stress and CV health.

Conclusion

The IPANAT is the first specific measure of implicit affect. The current two studies suggest that it is able to measure differences not only between affective responses to pictorial stimuli, as reported previously, but also between fear (with its positive subscale), anger and happiness as elicited using film clips (study 1). The findings suggest that the IPANAT is associated with CV activity during and after a stressor (study 2). Importantly, all findings for the IPANAT were independent of those for explicit affect, which were mostly absent.

Notwithstanding the remaining questions and limitations, these findings offer support for the theory that stress beyond self-report measures (i.e., unconscious stress-related cognition) at least partly relates to CV responses, that, when prolonged in daily life, are related to the progress and development of CV diseases. Especially because of this relevance for health, further research is needed to clarify the explanatory value of the IPANAT and possible other implicit measures of stress-related cognition, and their applicability to stress research.

Chapter 7

Automatic vigilance is associated with impaired cardiovascular recovery from recalling emotional memories

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Abstract

Self-report outcomes insufficiently explain the relationship between psychological stress and cardiovascular (CV) health. Implicit measures may provide new tools to assess the individuals' affective state beyond self-report and contribute to the understanding of processes outside of awareness in psychosomatic research. We tested whether an emotional Lexical Decision-making Task (LDT), as implicit measure of automatic vigilance, was related to slow CV recovery after a stress induction. Participants performed an angry (n = 24) or happy recall task (n = 30), followed by a self-report of their affective state and the LDT. This generated an index of automatic vigilance for negative (AVI-N) and positive information (AVI-P). CV activity was measured throughout the experiment. Lower self-reported happiness and a higher AVI-N were found in the anger recall condition compared with the happy recall condition. There were no differences in CV activity between conditions and the LDT subscales were not related to CV reactivity. However, irrespective of condition, higher AVI-N levels were associated with a generally higher diastolic blood pressure during recovery and lower AVI-P levels were associated with slower recovery of systolic blood pressure, heart rate, and total peripheral resistance. Importantly, self-reported affect was not related to CV reactivity or recovery. Thus, automatic vigilance for negative information is increased by an anger recall task and related to diastolic blood pressure recovery after emotional recall. Using the LDT as implicit measure of psychological stress could advance research on the relationship between psychological stress and CV disease by addressing processes outside of awareness.

Psychological stress, such as work stress, marital discourse, or worrying, has been related to an increased risk of the development or worsening of CV disease (e.g., 3,5,6,9,12,93,115, 263-266). Despite the abundance of research on this topic, the relationship remains poorly understood (e.g., 8,9). This may, partly, be a result of the methods to assess psychological stress, which are usually self-report measures of for example work stress, worry, anxiety, or negative affect (22-25,292,293). More specifically, self-report measures are likely to be insufficient since individuals may not (always) be capable of reflecting on their psychological state (e.g., 28,37). The amount of explained variance of CV responses to stressors based on self-report measures remains unsatisfactory (22-29,293). Moreover, part of the psychological stress response may occur outside of awareness, that is, beyond self-report, which could activate physiological responses that may lead to adverse consequences for one's health. This is referred to as unconscious stress (26,27). Thus, regarding the detrimental effects of psychological stress on CV health, the explanatory potential of measures beyond self-report has yet to be evaluated.

In various fields of psychology, measures have been developed to assess constructs beyond self-report, referred to as implicit measures. These measures assess psychological constructs that do not require deliberate processing by the individual (74), such as implicit stereotyping (66), affective evaluation (47), decision making (42), and job-related attitudes (81). In a previous study, we have use an implicit measure of affect, the Implicit Positive And Negative Affect Test (IPANAT; 28), which assesses affect through the process of affect infusion (see also 83,86). We found that the IPANAT subscales were related to the systolic blood pressure (SBP), heart rate variability (HRV), and total peripheral resistance (TPR) reactivity, and SBP and diastolic blood pressure (DBP) recovery, in addition to the self-report measure of affect after a mental arithmetic task (202). Although the IPANAT seems to be appropriate as implicit measure of affect, changes induced by psychological stress may be present at other levels of psychological processing as well, such as processing of emotional stimuli as shown in subliminal priming studies (e.g., 132,203). Here, we conducted a study with a different implicit measure, the Lexical Decision-making Task (LDT), which is believed to assess the cognitive activation of information of the construct measured (66,81,89). The LDT could be an appropriate measure of psychological stress outside of awareness, as it taps into the cognitive processes that are active at the moment of assessment, in other words, it should be able to assess negative affectivity induced by a stressor. Measuring the activation of this stress-related cognition and relating the outcomes to CV indices would not only indicate that implicit measures can provide additional information on psychological stress relative to CV activity, but would also support the idea of unconscious stress.

In a LDT, participants have to indicate whether a string of letters is a 'word' or a 'nonword' (81,89). Based on the construct of interest, construct-congruent and

incongruent responses are expressed in indexes of reaction times (RTs). Here, we used an emotional LDT in which the 'words' are negative, positive, or neutral. The accurate responses are averaged for all categories. The negative and positive average RTs are corrected for the neutral RTs, resulting in an automatic vigilance index for negative (AVI-N) and positive (AVI-P) information, respectively (90,314,315). Faster responses to one of these categories are thought to indicate greater neural accessibility (66). During a negative psychological state, such as psychological stress, the representation of negative information is activated. Consequently, accessibility of the negative concepts is enhanced, which would lead to a quicker perception and processing of the negative stimuli (e.g., 81). This has been found in relation to negative affective states such as anxiety and depression (316-321). Moreover, the LDT has been previously used as implicit measure of performance-related cognition after a cognitive challenging task (90) and was found to be related to the recovery of heart rate (HR), but not HRV. In that particular study, the word categories of the LDT were related to intelligence and positive characteristics, rather than to negative affectivity, and BP was not studied. Here, we will induce psychological stress to instigate the cognitive processing of negative information and relate the outcomes of the emotional LDT (i.e., using negative and positive words) with BP, HR, HRV, and TPR.

We used an anger recall task to induce psychological stress and measured the concurrent CV responses before, during, and after the recall. The procedure was based on a study by Gerin and colleagues (23) who found impaired CV recovery after the anger recall in participants with high levels of trait rumination. In general, anger recall procedures have been used to study the effect of psychological stress on CV activity (e.g., 93,322,323). However, these studies have not included a control for the anger induction part of the procedure and we cannot state with certainty that previous results are mainly due to the anger inducing aspect of the studies, rather than more general procedural (and perhaps also stressful) characteristics such as providing a stranger with personal information. Thus, we included a happy recall condition to control for these latter effects. Furthermore, impaired recovery from a stressor is thought to be most detrimental for health (e.g., 13,88). Therefore, we have focused on the course of the CV activity after the stressor. We expected an increase in SBP, DBP, HR, TPR, and a decrease in HRV during the task and an impaired recovery to baseline of these outcomes. Regarding the implicit measure, we expected that a higher AVI for negative affective information and a lower AVI for positive affective information would occur in the anger recall group, relative to the happy recall group, and that these responses would be related to increased CV reactivity and slower CV recovery, and that this relationship would be, at least partly, independent of self-reported affect.

Method

Participants

Participants (N = 61, M = 21.1, SD = 2.88) were recruited through an online registry system of Leiden University and received eight euro or course credits as a reward. Exclusion criteria were current (treatment of) psychological or/and CV health problems or/and the use of drugs that may influence CV activity. Participants were randomly assigned to the anger or happy recall condition as described below and provided informed consent before the start of the experiment. The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University (number 3145923676).

Instruments

As an implicit measure of psychological stress, a Dutch version of an emotional LDT (89) was provided. In the 64 trials, a string of letters was shown for 1000 ms and participants had to indicate as quickly and accurate as possible whether it constituted a 'word' or a 'nonword' on a keyboard. 'Words' consisted of eight positive (e.g., strong, intelligent), eight negative (e.g., unfair, hateful) words and 16 neutral words (e.g., sandwich, lamp), selected from the word-set of Hermans and De Houwer (255). All trials started with a fixation cross presented for 2000 ms and ended after a response. RTs and responses were recorded. For accurate responses only, outliers (>3*SD of the overall mean RT) were excluded and averages for stimulus type (positive, negative, neutral, or nonword) were calculated. Increased activation of negative and positive information was determined by subtracting mean RT of the negative and positive trials from the neutral trials, respectively. The resulting AVI's for negative (AVI-N) and positive (AVI-P) information indicated higher activation with larger values.

As self-report measure of affect, a VAS was provided to indicate the effect of the manipulation. Participants were asked to what extent they felt a certain emotion (e.g., 'How annoyed are you at this moment?') for twelve emotions (i.e., joyful, cheerful, happy, annoyed, irritated, angry, afraid, frightened, scared, sad, gloomy, unhappy). Answers were given on a horizontal line of 10 cm at the bottom of the screen, with zero indicating 'not at all' and 100 indicating 'very much'. Scores were averaged into self-reported anger, happiness, sadness, and fear, but only self-reported anger and happiness were used in the analyses. Cronbach's α's were sufficient with .83 for both self-reported anger and happiness.

Several questionnaires on trait and personality were provided to control for any group differences. The Dutch version of the State-Trait Anxiety Inventory, Trait version (STAI-T) was provided to test the tendency to experience all situations as threatening (280). This self-report questionnaires contains 20 items rated on a 4-point Likert scale and has a good internal consistency and validity (280). In this sample the Cronbach's α was high with .90. The Dutch version of the State-Trait Anger Expression Inventory,

trait version (324) was used to assess the tendency to display anger. The questionnaire contains 10 items rated on a 4-point Likert scale and has a good reliability and validity (324). In this sample, the Cronbach's α was high with.82. The inability or difficulty to name, and express emotions and the tendency to direct attention externally was measured with the Toronto Alexithymia Scale (TAS-20; 325). The scale has been shown to be valid (326) and in this sample the Cronbach's α was moderate with .64.

SBP and DBP (mmHg) were measured with the Portapres Model-2 (Finapres Medical Systems, Amsterdam, The Netherlands), which uses a noninvasive method to measure BP. To assess HR (bpm) the electrocardiogram was recorded with Kendall® 200 Covidien electrodes at a sample rate of 200 Hz with BIOPAC MP150 (Biopac Systems, Goleta, CA, USA). To monitor data quality during acquisition and correct artifacts Acqknowledge 3.9.1.4 was used. The data were extracted with a tailor made toolbox in Matlab R2012b, which applied a low-pass filter (20 Hz, Blackman 40 coefficients) to the BP signal, upsampled the electrocardiogram to 1000 Hz and applied a comb filter (50 Hz, Q = 5). For HRV (95) the root mean square successive differences (RMSSD; ms) were calculated from the interbeat intervals. TPR (mmHg.min/L) was calculated using an approximation of cardiac output (CO; 229,230,302) and mean arterial pressure (189). From these estimations only the outcome measure of interest, TPR, is reported. The CV outcome measures were obtained continuously and averages were calculated over the last min of baseline, the entire recall phase (M = 368.3 s, SD = 68.8), and the 15 min of recovery per min.

Procedure

After being welcomed into the lab, the participants were informed on the procedure and provided informed consent before starting with the experiment. The participant was seated in a separate room from the experimenter, who could monitor movements and behavior through a one-way mirror. First, demographics and biobehavioral variables were obtained, the CV measures were placed according to protocol, and the quality of the signals were checked. All further instructions were displayed on the monitor of a computer using E-Prime 2.0.8.90. The baseline was a five min period during which participants could read a magazine.

For the emotional recall procedure, in line with previous research (23), participants wrote down three emotional events that occurred during the previous year. In the anger recall condition, participants were asked to recall events that had upset them and made them angry. They were encouraged to choose events that were not completely solved and still evoked a lot of anger when thinking about it. In the happy recall condition, participants were asked to write down events that made them happy and cheery. The events should still elicit happiness when thinking about them. In both conditions participants had to rate the evens for the experienced anger or happiness on a 7 point Likert scale. The participants were then instructed to select one of these

memories to discuss with the experimenter. The experimenter went into the room when the participants indicated they were ready. The recall took about five min, allowing participants to finish thoughts beyond the specific time frame, during which they described in detail what happened, how it made them feel at the time, and their feelings at the time of the experiment on the issue. The experimenter did not show agreement or disagreement with the participant's statements, but merely nodded and maintained eye contact to encourage further elaboration. The experimenter left the room and the recovery started with a min during which participants did not perform any tasks and were instructed to remain seated for measurement purposes. After one min of recovery, the LDT and VAS were provided. Finally, the CV measures were detached and participants were debriefed.

Statistical analyses

Baseline differences between conditions in biobehavioral variables were analyzed with *t* tests and chi-square tests. Change scores were calculated for all physiological outcome measures to represent reactivity (238). The effect of the manipulation on the AVI's and VAS subscales and CV reactivity were analyzed with two-sided *t* tests and corrected for multiple comparison using the Benjamini Hochberg procedure (275-277), for which the false discovery rate was set at 10%. Effect sizes are expressed in *r* (239). Pearson correlations were calculated for the relationship between and amongst the AVI's and VAS subscales. Hierarchical multiple regression analyses were used to assess the relationship between condition, CV *reactivity*, and the AVI's and VAS subscales. Effect size was calculated using the spreadsheet by Lakens (182).

As main analyses, CV *recovery* was analyzed with multilevel modelling for all outcome measures separately (e.g., 279). Multilevel analyses (MLA) were used to assess the role of Condition (angry vs. happy) and the associations of AVI-N, AVI-P, VAS-anger, and VAS-happy with CV recovery throughout the 15 min of recovery (306). MLA it has various advantages over repeated measures ANOVAs when analyzing effects of time, such as a better handling of missing data and including individual slopes into the model and thus is able to consider multiple levels in the data (e.g., 279). The change in physiological responding over the 15 min was modelled with Condition, AVI-N, AVI-P, VAS-anger, and VAS-happy as predictors. CV baseline and reactivity were included as covariate in the model and were mean centered, as were AVI-N, AVI-P, VAS-anger, and VAS-happy. For each CV measure a separate model was built, but for all models Time was the level 1 variable (the measurements' course over 15 min) and Level 2 was the person level (all other predictors and covariates). 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).

Models were built in the following order. First, the basic growth model was fit, which included the covariates, to model change over time. Here, Time was used as

continuous predictor to model linear, quadratic, and cubic change (278). The best fitting covariance structure for the error variance was applied, which was either heterogeneous autoregressive, autoregressive, or diagonal, as is appropriate for fitting growth models (see for example 278). The additional value of BMI, smoking, gender, and relationship status was considered at this stage and included when it improved the model (based on the AIC and BIC). Finally, Condition was evaluated as a predictor, resulting in Model 1. Sequentially, we included AVI-N and AVI-P, and their interaction with Time and Time^2 (Model 2) to evaluate the association with CV recovery for each measure. In a similar way, the associations of CV recovery with VAS-anger and VAS-happy were evaluated (Model 3). Finally, all of these measures were added (Model 4) to evaluate the additional explanatory value of the implicit measure in addition to VAS subscales. The fit of each Model 2 and 3 was compared with Model 1. The fit of Model 4 was compared with one of the other models, depending on which had a better fit. All analyses were performed using SPSS 23.0.

Results[1]

Three participants were excluded based on the use of sympathomimetic drugs (salbutamol) and one participant was excluded due to extreme alcohol consumption (10 units in the 24 h before the experiment). In two cases, the experiment failed due to incorrect execution of the manipulation and in one case there were severe technical issues. The data for these participants were excluded from analyses. In several cases BP or HR recordings were of low quality, which led to smaller sample sizes for some

[1] We also performed a similar study, with the only difference being the implicit measure used. In this second study the LDT was replaced with the Morphing Faces Task (MFT, also known as the Facial Expression Recognition Task; 327). In this task, a series of faces morph from a neutral into a happy, angry, scared, or sad facial expression. Once participants recognize an emotion they stop the morph and have to identify the emotion. Faster responses to negative stimuli are thought to indicate an attentional bias, which has been found with the MFT in individuals with social anxiety and generalized anxiety disorders (e.g., 328,329). Antypa et al. (2011, 330) found that recent stressful events were related to earlier recognition of emotional expression of sadness and anger. It was expected that a faster recognition of negative emotion expressions and slower recognition of positive emotion expressions would occur in the angry recall condition, compared with happy recall condition, and that these responses were related to increased CV reactivity and slower recovery of CV responses in addition to self-reported affect. The final sample (N = 48, age M = 21.2, SD = 2.29, 79.2 % female) was randomly assigned to the angry (n = 29) or happy (n = 19) recall condition. However, the different subscales of the MFT (sad, anger, fear, happiness) were highly correlated (r = .99), there were no differences between conditions (ts < .50, ps> .70), and no relationship with CV reactivity or recovery was apparent (rs < .25, p > .05). This led to the conclusion that the task as executed here (see 330), was not a valid measure for this purpose and was considered inappropriate for measuring psychological stress beyond self-report.

outcome measures. Two participants showed deviating levels of RMSSD (> 3*SD) for which the data were considered to be missing at random. A square root transformation and a log transformation were applied to RMSSD and TPR, respectively. Furthermore, one outlier was found for the AVI-N for which the data were also labelled as missing and considered to be random. Finally, RT data of two participants with an accuracy below 70% were excluded. The final sample (N = 54) had a mean age of 21.1 (SD = 2.96) and consisted of 42 females (77.8 %). There were no differences between the angry (n = 24) and happy (n = 30) recall condition in baseline characteristics, see Table 1, except that in the anger recall condition more participants were in a relationship, compared with the happy recall condition. However, when checking for this variable in the main analyses it did not show any significant contribution to the model.

Self-reported affect and automatic vigilance

After the manipulation, in the anger recall condition VAS-anger (M = 19.2, SD = 19.7) was higher compared with the happy recall condition (M = 9.52, SD = 9.63), but this was not statistically significant, Mann-Whitney U = 256, Z = 1.81, p = .070, r = .25. However, VAS-happy was significantly lower in the anger recall condition (M = 64.7, SD = 15.1) compared to the happy recall condition (M = 74.4, SD = 11.6), $t(52)$ = 2.69, p = .010, r = .35. Furthermore, in the anger recall condition the AVI-N (M = 29.1, SD = 29.4) was larger compared to the happy recall condition (M = 3.34, SD = 50.0), $t(47.8)$ = -2.31, p = .025, r = .32 (corrected dfs since the equality of the variances could not be assumed). The AVI-P did not statistically differ between conditions (anger: M = 28.7, SD = 38.3; happy: M = 11.8, SD = 53.7, $t(50)$ = -1.26, p = .215, r = .18).

In addition, no statistically significant associations were found between the AVI's and the VAS subscales, rs < .30, ps > .05, and between the AVI's, $r(51)$= .27, p = .055, but a strong negative relationship was found between VAS-anger and VAS-happy, $r(54)$ = -.67, p < .001.

Cardiovascular reactivity

In both conditions changes from baseline during the manipulation were evident for SBP (ΔM = 14.0, SD = 10.7, $t(44)$ = 8.77, p < .001, r = .80), DBP (ΔM = 7.41, SD = 4.79, $t(44)$ = 10.4, p < .001, r = .84), HR (ΔM = 5.45, SD = 4.13, $t(51)$ = 9.51, p < .001, r = .80), and TPR (ΔM = -0.033, SD = 0.06, $t(44)$ = -6.20, p < .001, r = .68), but not for RMSSD (ΔM = 0.153, SD = 0.994, $t(49)$ = 1.09, p = .28, r = .15).

However, we found no statistical support for any differences between the conditions on SBP reactivity (anger recall: ΔM = 13.4, SD = 12.7; happy recall: ΔM = 14.4, SD = 9.36, $t(43)$ = -0.299, p = .77, r = .05), DBP reactivity (anger recall: ΔM = 6.82, SD = 5.09, happy recall: ΔM = 7.80, SD = 4.64, $t(43)$ = -0.669, p = .51, r = .10), HR reactivity (anger recall: ΔM = 4.81, SD = 3.32, happy recall: ΔM = 5.96, SD = 4.67, $t(50)$ = -0.992, p = .35, r = .14), RMSSD reactivity (anger recall: ΔM = 0.261, SD = 0.911, happy recall: ΔM =

0.062, *SD* = 1.07 *t*(48) = 0.701, *p* = .49, *r* = .10), and TPR reactivity (anger recall: Δ*M* = -0.030, *SD* = 0.034, happy recall: Δ*M* = -0.034, *SD* = 0.037 *t*(43) = 0.394, *p* = .70, *r* = .06).

TABLE 1 Biobehavioral characteristics stratified by condition

	Angry (*n* = 24)			**Happy (*n* = 30)**			
Measure	*M*	*SD*	*n*	*M*	*SD*	*n*	t/χ^2
Demographics							
Age, years	20.7	2.53	24	21.4	3.28	30	-0.89
Female sex[1]	18	(75)	24	24	(80)	30	0.19
BMI	22.0	3.07	24	22.2	2.65	30	-0.20
Dutch nationality[1]	24	(100)	24	30	(100)	30	n.s.
Biobehavioral variables							
Smoking[1]	4	(17)	24	2	(7)	30	1.35
Caffeine (test day)	0.21	0.51	24	0.27	0.58	30	-0.39
Alcohol use (glass/last 24h)	0.75	1.78	24	0.38	1.60	26	0.77
Relationship[1]	14	(58)	24	10	(33)	30	3.38^{+}
Cardiovascular measures							
SBP	128.0	18.1	21	124.4	14.2	28	0.77
DBP	68.7	11.0	21	67.3	10.6	28	0.46
HR	78.5	12.6	24	76.0	11.4	29	0.77
RMSSD[2]	35.4	19.7	24	39.6	22.3	27	-0.62
TPR[3]	10.0	2.81	21	10.3	2.30	28	-0.51
Personality							
Trait anxiety	38.3	8.63	24	37.1	9.96	29	0.46
Trait anger[4]	17.5	3.80	22	16.0	3.99	28	1.38
Alexithymia	47.0	7.56	22	48.1	7.19	28	-0.57

Note. There were no significant differences between conditions. *Abbreviations*: BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

[1] Displayed are the number of positive responses (percentage). The Pearson χ2 was used as test statistic.

[2] RMSSD was square root transformed. Untransformed *M*s and *SD*s are displayed.

[3] TPR was logarithmic transformed. Untransformed *M*s and *SD*s are displayed.

[4] One participant in the anger condition was excluded for the variable of trait anger, which normalized the data

CV reactivity and affect

To test the hypothesis that CV reactivity would be related to AVI-N and AVI-P, hierarchical regression analyses were conducted for each CV reactivity measure. In all the models condition was added at step 1 and VAS subscales at step 2. Since we expected that automatic vigilance would explain CV activity over and above VAS subscales, the

AVI's were added in step 3. Self-reported NA and PA were highly correlated, $r(54)= -.665$, $p < .001$, but VIF and tolerance were of acceptable levels in all tests and thus the assumption of multicollinearity was not violated (308). Adding BMI, smoking status, gender, or relationship status did not improve the model fit, ΔR^2s< .10, ps > .05, and models are reported without these variables. Changes in CV reactivity were not related to the AVI'S or VAS subscales, ΔR^2s <.15, ps > .10, see Table 2.

CV recovery

To model SBP recovery, a heterogeneous autoregressive covariance structure was applied to the error variance. The slope of Time was allowed to vary randomly between participants. Adding Condition to the Model did not improve the fit, nor did the addition of any of the covariates. Results are displayed in Table 3. There were significant associations of Time as well as Time^2, indicating that the recovery slope was composed of a linear decrease as well as a quadratic change (Model 1). The latter represented a trend with the fastest decrease at the beginning and a (small) increase in SBP towards the end of the recovery phase. The addition of AVI-N and AVI-P and their interactions with Time (Model 2) improved the model, $\Delta AIC = 183.7$ and $\Delta BIC = 167.0$, compared with Model 1. AVI-P×Time was positively associated with the recovery of SBP ($B = 0.005$, $t(41.8) = 2.13$, $p = .040$). Adding VAS-anger and VAS-happy and their interactions with Time to the model without the AVI's (Model 3) did not improve the model fit, $\Delta AIC = -1.6$ and $\Delta BIC = -19.5$, compared with Model 1, nor did a combination of the AVI's and VAS subscales (Model 4; $\Delta AIC = -2.3$ and $\Delta BIC = -20.2$) compared with Model 2. Thus, Model 2 provided the best fit to the data and indicates that a lower AVI-P was related to a slower linear decrease of SBP during recovery, that is slower recovery, but AVI-N and VAS subscales were not related to recovery of SBP.

To model DBP recovery, an autoregressive covariance structure was applied to the error variance. The slope of Time was allowed to vary randomly between participants. Adding Condition to the Model did not improve the fit, nor did the addition of any of the covariates. Results are displayed in Table 4. There was a significant association of Time, indicating that the recovery slope was composed of a linear decrease (Model 1). When adding AVI-N and AVI-P and their interactions with Time to the model (Model 2) the model fit improved, $\Delta AIC = 126.9$ and $\Delta BIC = 109.5$. Recovery of DBP was positively associated with AVI-N ($B = 0.04$, $t(37.9) = 2.75$, $p = .009$) and tended to be negatively associated with AVI-N×Time (but not statistically significantly so), $B = -0.002$, $t(42.8) = -1.95$, $p = .058$. Adding VAS-anger and VAS-happy and their interactions with Time to the model without the AVI's (Model 3) did not improve the model fit, $\Delta AIC = -5.8$ and $\Delta BIC = -23.8$, compared with Model 1, nor did a combination of the AVI's and VAS subscales compared with Model 2 (Model 4; $\Delta AIC = -6.5$ and $\Delta BIC = -24.4$). Thus, Model 2 provided the best fit to the data and indicated that, in contrast to our hypothesis,

TABLE 2 Summary of hierarchical multiple regressions for the CV change scores during the emotion recall procedure

	SBP (mmHg)[a]			DBP (mmHg)[a]			HR (bpm)[b]			RMSSD (ms)[c]			TPR (mmHg.min/L)[a,d]		
	B	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	***β***
Constant	46.1**	14.2		21.0**	6.46		4.67	5.01		-1.46	1.25		-0.05	0.05	
Condition	-4.41	3.72	-.20	-2.38	1.70	-.24	-1.63	1.38	-.19	0.41	0.35	.21	0.01	0.03	.13
Self-reported anger	-0.22	0.15	-.30	-0.08	0.07	-.24	0.03	0.05	.12	0.008	0.01	.12	0.0002	0.001	.09
Self-reported happiness	-0.41*	0.18	-.53	-0.17	0.08	-.50	0.01	0.06	.04	0.02	0.01	.29	0.0002	0.001	.09
AVI-N	0.04	0.04	.14	0.006	0.02	.05	-0.02	0.02	-.19	-0.003	0.004	.12	-0.0001	0.0001	-.17
AVI-P	0.03	0.04	.14	0.02	0.02	.18	0.02	0.01	.23	-0.001	0.003	.07	-0.0001	0.0001	-.13
F		1.34			1.10			1.05			0.82			0.44	
R^2		.15			.13			.11			.06			.06	
ΔR^2		.04			.03			.06			.02			.05	

Note. The table shows the associations between condition, affect, and CV change scores as generated by the final model (step 3); Condition was added at step 1, self-reported NA and PA at step 2, and AVI-N and AVI-P at step 3 to indicate the additional value of the implicit measure**.** The *F* statistic refers to that of the final model. $\boldsymbol{\Delta R^2}$ is the difference in explained variance between step 2 and step 3, which represents the additional variance of the change scores explained by AVI-N and AVI-P. *Abbreviations*: AVI-N = Automatic vigilance index for negative words, AVI-P = Automatic vigilance index for positive words, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, RMSSD = Root mean square of successive differences, TPR = Total peripheral resistance.

[a] $N = 43$.

[b] $N = 49$.

[c] $N = 47$, a square root transformation was applied.

[d] A logarithmic transformation was applied.

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

a generally higher DBP during recovery was related to *higher* AVI-N. VAS subscales and AVI-P were not related to recovery of DBP.

To model HR recovery, an autoregressive covariance structure was applied to the error variance. The slope of Time was allowed to vary randomly between participants. Adding Condition to the Model did not improve the fit, nor did the addition of any of the covariates. Results are displayed in Table 5. There was a significant association of Time, Time^2, and Time^3 indicating that the recovery slope was composed of a general linear decrease, but also of a quadratic and cubic change (Model 1). Adding AVI-N and AVI-P and their interactions with Time (Model 2) improved the model, Δ*AIC* = 241.8 and Δ*BIC* = 224.1, compared with Model 1. AVI-P×Time was positively associated with the recovery of HR, *B* = 0.002, *t*(47.1) = 2.36, *p* = .022. Adding VAS-anger and VAS-happy and their interactions with Time to the model without the AVI's (Model 3) did not improve the model fit, Δ*AIC* = -0.4 and Δ*BIC* = -18.9, compared with Model 1, nor did a combination of the AVI's and VAS subscales (Model 4; Δ*AIC* = -3.6 and Δ*BIC* = -21.9) compared with Model 2. Thus, Model 2 provided the best fit to the data and indicated that a lower AVI-P was related to a slower recovery of HR during recovery, which is consistent with our hypothesis, and the VAS subscales were not related to recovery of HR.

To model recovery of RMSSD, a heterogeneous autoregressive covariance structure was applied to the error variance. The slope of Time was allowed to vary randomly between participants. Adding Condition to the Model did not improve the fit, nor did the addition of any of the covariates. Results are displayed in Table 6. There was a significant association of Time and Time^2, indicating that the recovery slope was composed of a general linear decrease and a quadratic change (Model 1). The addition of AVI-N and AVI-P and their interactions with Time to the model (Model 2) compared with Model 1 did improve the model fit, Δ*AIC* = 98.5 and Δ*BIC* = 81.8, but no individual predictors were significantly related to recovery of RMSSD. Adding VAS-anger and VAS-happy to the model without the AVI's (Model 3) did not improve model fit compared to Model 1, Δ*AIC* = -6.2 and *BIC* = -24.6, nor did the combined addition of the AVI's and VAS subscales compared to Model 2 (Model 4: Δ*AIC* = -6.8 and *BIC* = -25). Thus, although Model 2 provided the most optimal model fit for the data, the predictors were not significantly related to the recovery of RMSSD.

Finally, to model recovery of TPR, an autoregressive covariance structure was applied to the error variance. The slope of Time was allowed to vary randomly between participants. Adding Condition to the Model did not improve the fit, nor did the addition of any of the covariates. Results are displayed in Table 7. There was a significant association of Time and Time^2, indicating that the recovery slope was composed of a general linear decrease and a quadratic change (Model 1). The model fit improved when AVI-N and AVI-P and their interactions with Time were added (Model 2) compared with Model 1, Δ*AIC* = 142.4 and Δ*BIC* = 159.8. Recovery of TPR was

positively associated with AVI-N, $B = 0.0003$, $t(42.0) = 2.13$, $p = .039$, and negatively with AVI-P×Time, $B = -0.00002$, $t(42.9) = -2.42$, $p = .020$. Adding VAS-anger and VAS-happy and their interactions with Time to the model without the AVI's (Model 3) slightly improved the model fit, $\Delta AIC = 1$ and $\Delta BIC = 19.1$, compared with Model 1. Recovery of TPR was positively (but not statistically significantly) associated with VAS-anger, $B = 0.0007$, $t(43.5) = 1.68$, $p = .099$. Furthermore, a combination of the AVI's and VAS subscales provided a slightly better fit compared with Model 2 (Model 4; $\Delta AIC = 0.9$ and $\Delta BIC = 18.8$). In this model 4, recovery of TPR was negatively related with AVI-P×Time, $B = -0.00002$, $t(42.9) = -2.31$, $p = .026$. The relationship with AVI-N was no longer statistically significant. Thus, Model 4 provided the most optimal fit to the data and indicates that participants with a lower AVI-P showed a slower recovery of TPR.

To summarize, for none of the CV outcome variables the model was improved by Condition, that is, there were no differences between the conditions in the slope of recovery as indicated by increasing, rather than decreasing, AIC's (Δ [0.31;73.9]) and BIC's (Δ [1.5;78.3]) compared to Model 1 for all variables. The basic models were most optimal when including the CV baseline and reactivity, that is, baseline and reactivity were predictive of the CV recovery slopes. Regarding the AVI's, across both conditions, lower levels of AVI-P were related to a slower recovery of, as indicated by significant AVI-P×Time interactions for SBP ($B = 0.005$, $t(40.7) = 2.13$, $p = .040$), HR ($B = 0.002$, $t(47.1) = 2.36$, $p = .022$), and TPR ($B = -0.00002$, $t(42.9) = -2.31$, $p = .026$). A higher AVI-N was only related to a generally higher DBP during recovery ($B = 0.04$, $t(37.9) = 2.75$, $p = .009$). See Figure 1 and 2. Notably, the VAS subscales were not related to CV recovery.

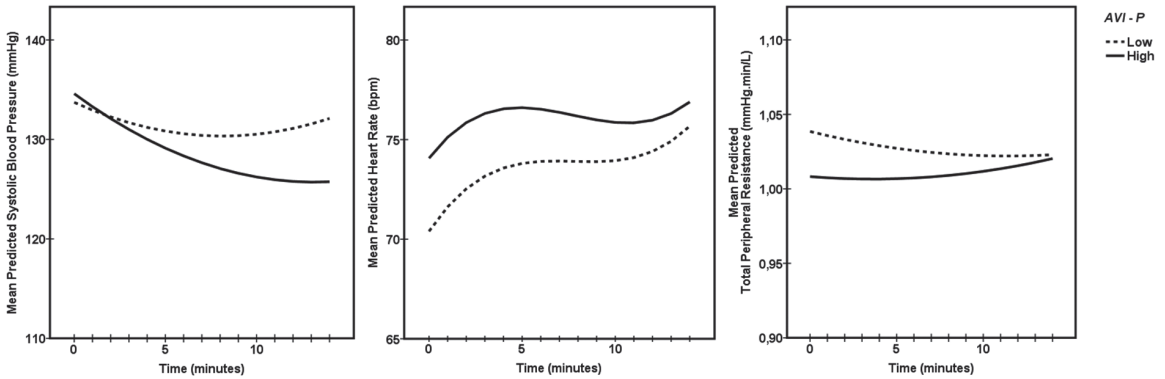


FIGURE 1 Mean predicted values of SBP, HR, and TPR over each of the 15 min of recovery from the emotion recall procedure displayed for low and high automatic vigilance for positive information (AVI-P), which was dichotomized for display purposes

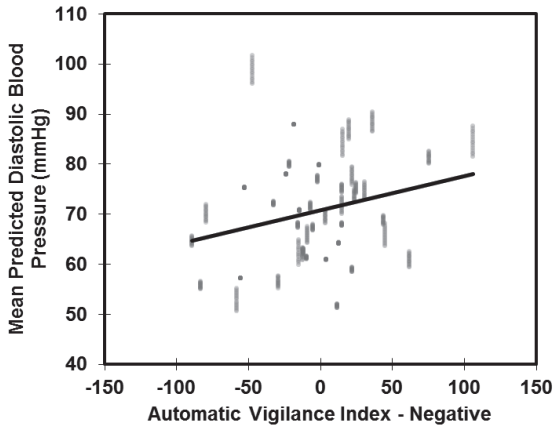


FIGURE 2 Mean predicted DBP during recovery in relationship with mean automatic vigilance for negative information (AVI-N). The grey spots display the raw values of the AVI-N to indicate the spread

TABLE 3 Summary of multilevel analysis for recovery of SBP (mmHg)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	135.2	1.57	86.2***	135.0	1.55	87.1***	135.4	1.48	91.7***	135.1	1.46	92.7***
Time	-1.05	0.29	-3.63***	-1.09	0.30	-3.42***	-1.10	0.29	-3.81***	-1.12	0.30	-3.76***
Time^2	0.05	0.02	2.71**	0.05	0.02	2.79**	0.05	0.02	2.88**	0.05	0.02	2.88**
Baseline SBP	0.80	0.08	10.7***	0.83	0.08	10.9***	0.84	0.07	11.5***	0.87	0.07	11.8***
Reactivity SBP	0.37	0.11	3.34**	0.38	0.11	3.42**	0.39	0.11	3.51**	0.41	0.11	3.68***
AVI-N				0.04	0.04	1.12				0.05	0.03	1.38
AVI-P				-0.03	0.03	-0.98				-0.03	0.03	-1.00
Time×AVI-N				-0.003	0.003	-0.95				-0.003	0.003	-1.02
Time×AVI-P				0.005	0.002	2.13*				0.005	0.002	1.87+
VAS-anger							-0.30	0.12	-2.42*	-0.25	0.12	-2.10*
VAS-happy							-0.17	0.13	-1.34	-0.07	0.12	-0.54
Time×VAS-anger							0.02	0.01	1.86+	0.01	0.01	1.42
Time×VAS-happy							0.02	0.01	1.60	0.008	0.01	0.68
AIC		4275.2			4091.5			4276.8			4093.8	
BIC		4383.6			4216.6			4403.1			4236.8	
N		24			28			28			32	

Note. Error at Level-1 was organized with a heterogeneous autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. When interactions with Time^2 were added to Model 2 and 3, the Models did not provide a better fit and the interactions were omitted from Model 3 and 4. Model 2 shows the best fit and was used to interpret the data. *Abbreviations*: SBP = Systolic blood pressure, AVI-N = Negative automatic vigilance index, AVI-P = Positive automatic vigilance index, VAS = Visual analogue scale, 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 for recovery of DBP (mmHg)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*T*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	71.5	0.62	116.0***	71.7	0.59	121.5***	71.5	0.61	117.9***	71.7	0.58	123.1***
Time	-0.09	0.05	-1.99+	-0.10	0.05	-2.16*	-0.09	0.05	-2.01+	-0.10	0.05	-2.19*
Baseline DBP	0.90	0.05	19.3***	0.88	0.05	19.0***	0.90	0.05	19.3***	0.89	0.05	19.1***
Reactivity DBP	0.51	0.11	4.85***	0.52	0.10	5.00***	0.51	0.11	4.68***	0.51	0.11	4.81***
AVI-N				0.04	0.02	2.75**				0.04	0.02	2.75**
AVI-P				-0.02	0.01	-1.29				0.01	0.01	1.00
Time×AVI-N				-0.002	0.001	-1.95+				-0.002	0.001	-1.91+
Time×AVI-P				0.001	0.001	1.49*				0.001	0.001	1.14
VAS-anger							-0.06	0.06	-1.05	-0.06	0.06	-1.08
VAS-happy							-0.07	0.06	-1.12	-0.05	0.06	-0.74
Time×VAS-anger							0.005	0.004	1.19	0.004	0.004	1.02
Time×VAS-happy							0.006	0.004	1.41	0.004	0.005	0.86
AIC		3095.4			2968.5			3101.2			2975.0	
BIC		3136.0			3026.5			3159.8			3050.9	
N		9			13			13			17	

Note. Error at Level-1 was organized with an autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. Model 2 shows the best fit and was used to interpret the data. *Abbreviations*: DBP = Diastolic blood pressure, AVI-N = Negative automatic vigilance index, AVI-P = Positive automatic vigilance index, VAS = Visual analogue scale, 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 5 Summary of multilevel analysis for recovery of HR (bpm)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*T*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	72.8	0.49	107.0***	72.8	0.49	148.5***	72.8	0.48	150.7***	72.9	0.49	148.7***
Time	1.30	0.23	5.58***	1.32	0.24	5.48***	1.30	0.23	5.59***	1.31	0.24	5.47***
Time^2	-0.18	0.04	-4.62***	-0.18	0.04	-4.52***	-0.18	0.04	-4.63***	-0.18	0.04	-4.52***
Time^3	0.008	0.002	4.21***	0.008	0.002	4.14***	0.008	0.002	4.22***	0.008	0.002	4.14***
Baseline HR	0.94	0.03	27.6***	0.94	0.04	26.5***	0.94	0.03	27.5***	0.94	0.04	26.4***
Reactivity HR	0.48	0.10	5.03***	0.48	0.10	4.90***	0.48	0.10	4.99***	0.48	0.10	4.88***
AVI-N				0.0009	0.01	0.10				-0.0003	0.01	-0.03
AVI-P				-0.01	0.009	-1.41				-0.01	0.01	-1.18
Time×AVI-N				0.0005	0.001	0.52				0.0003	-0.001	0.34
Time×AVI-P				0.002	0.001	2.36*				0.002	-0.001	1.77+
VAS-anger							-0.03	0.03	-0.80	-0.02	0.04	-0.667
VAS-happy							-0.03	0.04	-0.73	-0.02	0.04	-0.48
Time×VAS-anger							0.009	0.003	2.74**	0.007	0.003	2.15*
Time×VAS-happy							0.009	0.004	2.46*	0.007	0.004	1.65
AIC		3991.7			3749.9			3992.1			3753.5	
BIC		4042.9			3818.8			4061.8			3840.7	
N		11			15			15			19	

Note. Error at Level-1 was organized with a heterogeneous autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. When interactions with Time^2 and Time^3 were added to Model 2 and 3, the Models did not provide a better fit and the interactions were omitted from Model 3 and 4. Model 2 shows the best fit and was used to interpret the data.
Abbreviations: HR = Heart rate, AVI-N = Negative automatic vigilance index, AVI-P = Positive automatic vigilance index, VAS = Visual analogue scale, 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 for recovery of HRV (RMSSD, ms)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*T*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	6.42	0.10	63.4***	6.40	0.10	66.4***	6.42	0.10	63.3***	6.40	0.10	66.1***
Time	-0.08	0.03	-2.88**	-0.07	0.03	-2.70**	-0.08	0.03	-2.87**	-0.07	0.03	-2.68**
Time^2	0.003	0.002	1.94+	0.003	0.002	1.67+	0.003	0.002	1.92+	0.003	0.002	1.67+
Baseline HRV	0.88	0.04	20.4***	0.92	0.04	24.2***	0.88	0.04	20.6***	0.92	0.04	24.4***
Reactivity HRV	0.27	0.08	3.62***	0.35	0.07	5.19***	0.28	0.08	3.74***	0.36	0.07	5.29***
AVI-N				-0.002	0.002	-1.16				-0.002	0.002	-1.23
AVI-P				0.0003	0.002	0.21				0.0005	0.002	0.26
Time×AVI-N				0.00002	0.0003	0.11				0.00002	0.0003	0.07
Time×AVI-P				-0.0001	0.0002	-0.66				-0.00008	0.0002	-0.40
VAS-anger							-0.001	0.007	-0.20	0.002	0.007	0.25
VAS-happy							-0.002	0.008	-0.20	-0.001	0.008	-0.12
Time×VAS-anger							-0.0006	0.0008	-0.83	-0.0004	0.0008	-0.50
Time×VAS-happy							-0.0008	0.0008	-0.88	-0.0006	0.001	-0.60
AIC		1546.3			1447.8			1552.5			1454.6	
BIC		1657.0			1575.2			1681.6			1600.2	
N		24			28			28			32	

Note. Error at Level-1 was organized with a heterogeneous autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. When interactions with Time^2 were added to Model 2 and 3, the Models did not provide a better fit and the interactions were omitted from Model 3 and 4. Model 2 shows the best fit and was used to interpret the data. RMSSD was square root transformed. *Abbreviations*: HRV = Heart rate variability, RMSSD = Root mean square successive differences, AVI-N = Negative automatic vigilance index, AVI-P = Positive automatic vigilance index, VAS = Visual analogue scale, 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 7 Summary of multilevel analysis for recovery of TPR (mmHg.min/L)

	Model 1			Model 2			Model 3			Model 4		
Predictor	*B*	*SE*	*t*	*B*	*SE*	*T*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	-0.76	0.10	-7.74***	-0.73	0.10	-7.53***	-0.77	0.09	-8.27***	-0.73	0.09	-8.07***
Time	-0.002	0.001	-1.76^{+}	-0.002	0.001	-1.78^{+}	-0.002	0.001	-1.77^{+}	-0.002	0.001	-1.79^{+}
Time^2	0.0001	0.00007	1.95^{+}	0.0001	0.00007	1.86^{+}	0.0001	0.00007	1.95^{+}	0.0001	0.00007	1.85^{+}
Baseline TPR	0.89	0.05	18.4***	0.87	0.05	18.2***	0.89	0.05	19.2***	0.88	0.05	19.4***
Reactivity TPR	0.27	0.08	3.62***	0.54	0.14	3.98***	0.51	0.13	3.81***	0.53	0.13	4.19***
AVI-N				0.0003	0.0001	2.13*				0.0002	0.0001	1.81^{+}
AVI-P				0.00007	0.0001	0.62				0.0001	0.0001	1.21
Time×AVI-N				-0.00001	0.00001	-1.11				-0.00001	0.00001	-0.98
Time×AVI-P				-0.00002	0.00001	-2.42*				-0.00002	0.00001	-2.31*
VAS-anger							0.0007	0.0004	1.68^{+}	0.0005	0.0004	1.18
VAS-happy							-0.00001	0.0005	-0.20	-0.0004	0.0005	-0.96
Time×VAS-anger							-0.00005	0.00004	-1.33	-0.00004	0.00004	-0.96
Time×VAS-happy							-0.00004	0.00004	-1.01	-0.000008	0.00004	-0.20
AIC		-2967.7			-2825.3			-2966.7			-2824.4	
BIC		-2922.6			-2762.8			-2903.5			-2744.0	
N		10			14			14			18	

Note. Error at Level-1 was organized with an autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centred. When interactions with Time^2 were added to Model 2 and 3, the Models did not provide a better fit and the interactions were omitted from Model 3 and 4. Model 4 shows the best fit and was used to interpret the data. TPR was log transformed. *Abbreviations*: TPR = Total peripheral resistance, AVI-N = Negative automatic vigilance index, AVI-P = Positive automatic vigilance index, VAS = Visual analogue scale, 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

This study set out to test whether a measure beyond self-report of psychological stress, that is, an implicit measure, should be employed to add explanatory power in the psychological stress and CV responses relationship. We explored the potential explanatory role of the emotional LDT as implicit measure of psychological stress in CV recovery from a stressor. As the LDT is assumed to measure automatic vigilance, representing the cognitive activation of information (66,81,89), higher levels of automatic vigilance for negative information (the AVI-N index) and lower levels of automatic vigilance for positive information (the AVI-P index) would indicate psychological stress beyond self-report and relating these findings to CV activity may provide support for the unconscious stress hypothesis. The findings indicate that the CV activity during and after an anger recall procedure to induce psychological stress, as well as after a happy recall procedure as control condition, was related to AVI-N, AVI-P, and self-reported affect. During the recall task, SBP, DBP, and HR increased, and TPR decreased relatively to the baseline, but HRV did not. In other words, in contrast to the expectations there were no differences in CV reactivity between conditions. Automatic vigilance for negative, but not for positive, information was stronger in the anger recall condition compared with the happy recall condition, but the implicit measure was not related to CV reactivity. CV recovery was also similar across conditions. However, stronger automatic vigilance for negative information was found to be related to a general higher DBP during recovery, but not to change over time. It was not related to SBP, HR, HRV, or TPR. In contrast, lower automatic vigilance for positive information was related to a slower recovery of SBP, HR, and TPR, but not to recovery of DBP or HRV. Importantly, self-reported affect was not related to the CV reactivity nor to recovery, although it was related to condition in the expected direction, that is, more negative affect (albeit statistically nonsignificant) and less positive affect after the anger condition compared to the control condition. Thus, the emotional LDT appears to detect cognitive processes related to stress-induced CV recovery in addition to self-report. We will discuss these results in more detail below.

In contrast to our expectations, we did not find differences in CV reactivity and recovery between conditions. Both conditions elicited an increase in SBP, DBP, and HR, and a decrease in TPR and the level of reactivity was related to the progress of recovery. Previous studies did not include a control condition (e.g., 23,93,322,323) and based on the current findings it can now be questioned whether it was the induced anger (or reduced positive affect for that matter) or the procedure itself that elicited changes in CV reactivity. This is further stipulated by the absence of a statistical significant difference between conditions in self-reported anger. On the other hand, these findings are similar to those in our previous study (202), in which there was also an absence of CV differences between a standard anger-induction procedure (math with harassment), and a logical control (math without harassment)

that previous studies using this anger provocation did not use. These findings call for more rigorous and consistent use of control conditions in psychosomatic research of the physiological effects of psychological stress.

Furthermore, we expected an increase but found a decrease of TPR throughout the experiment. An increase in TPR is assumed to represent threat, which one would expect to occur in response to a stressor (99,100). The decrease of TPR would then indicate challenge, meaning that the participants are likely to have experienced the emotional recall procedure as a challenge. One explanation is the nature of the sample, which consisted mainly of psychology students who may well be fond of discussing emotional content and could try to do this to their best effort, but it may also be that the study information provided in advance reduced the threatening nature of the manipulation. To our knowledge, TPR in relation with psychological stress has not been previously addressed with an anger recall procedure and the current findings should therefore be replicated in a similar but also in different samples. The absence of an effect on HRV reactivity is also in line with our previous study (202), which may indicate that in this healthy sample participants could regulate their affective state adequately. This is further stipulated by the relationship of AVI-P with SBP, HR, and TPR, as we will discuss below.

In line with our expectations, differences in self-reported affect was apparent in the anger recall condition compared with the happy recall condition, that is, participants reported more, statistically nonsignificant, self-reported anger and less self-reported happiness. However, these findings were not related to CV activity and may be the result of procedural characteristics of the study. Furthermore, in line with our expectations automatic vigilance for negative, but not for positive, information was higher in the anger recall condition compared with the happy recall condition. Importantly, this activation of negative information was related to elevated DBP during recovery. However, activation of positive information was found to be related to faster recovery of SBP, HR, and TPR. As there was no effect of condition on this positive subscale of the LDT, it seems likely that a general activation of AVI-P was present that resulted in adaptive CV responses to psychological stress. A similar effect of positive affectivity measured at an implicit level has been found earlier in studies with the IPANAT, where it was related to a lower cortisol excretion and faster CV recovery (85,202). All in all, these findings suggest that implicit measures, both the negative and positive indexes, explain CV recovery in addition to self-reported affect and stipulate the additional value of implicit measures in CV stress research. Implicit measures such as the LDT and IPANAT appear to able to detect parts of the *core* affect (310) that are not necessarily reflected by self-report but may ultimately be co-determinants in the etiology of CV disease.

Some limitations of this study have to be considered. First, no baseline measurement was performed for the self-report and implicit measures. Consequently, no firm

statements can be made on pre-existing psychological states and their influence on the current findings. However, these measurements were intentionally left out to prevent carry-over effects on the measures and possible priming effects of the emotion-related words, but further studies on the validation of the LDT would need to include a baseline measure. Second, the duration of the LDT was on average five min, but the recovery phase lasted 15 min. This means that participants had to sit and wait quite some time for the recovery phase to be finished. Despite our efforts to make sure that the participants were comfortable, this may have led to, for example, boredom. Finally, since participants chose the situation they wanted to tell the experimenter, it could be that they did not pick out the situation that elicited the strongest emotions. In an effort to respect the privacy of the participants, the emotion recall may not have been as strong as expected.

Conclusion

The current study suggests that the LDT, measuring automatic vigilance, is related to CV recovery after an emotional recall procedure to induce psychological stress, in addition to self-reported affect. Not only do these findings emphasize the prospective explanatory capabilities of implicit measures, it also highlights the role of processes outside of awareness in CV activity which may negatively contribute to the worsening or development of CV disease and provides further evidence for the unconscious stress hypothesis.

Chapter 8

General Discussion

The aim of this thesis was to examine the association of stress-related cognition outside of awareness with cardiovascular (CV) activity. As elaborated on in the Introduction, studies on the relationship between psychological stress and CV disease have not been able to fully explain how this negative psychological state may result in a prolonged adverse physiological state (8,9, 22-25). One possible explanation is the occurrence of stress-related cognitions outside of awareness, that is, unconscious stress, which may activate and even prolong physiological stress-responses (26-28). This thesis represents a first attempt to examine the role of processes outside of awareness in CV responses to stressors through a systematic review and a series of experiments, described in the previous six chapters. In this last Chapter these findings are first briefly summarized and then discussed in terms of relevant theoretical and methodological issues. The reader will also find a description of the strengths and limitations of the studies in this thesis, and the possible future of the unconscious stress hypothesis is portrayed.

Main findings

Unconscious processes and their relationship with physiological activity have not been described in the context of psychological stress. However, a vast amount of studies have been performed using methods to present stimuli outside of awareness of the participants while measuring peripheral physiological activity (e.g., 26,27). In order to attain accumulation of knowledge on this topic, a systematic review was performed (**Chapter 2**). This review included 65 experimental studies in which negative affective stimuli versus control stimuli were presented outside of awareness. In other words, the stimuli that were presented did not require deliberate processing. During this procedure peripheral physiological responses (i.e., CV, electrodermal, electromyographical, hormonal, and immunological parameters) were recorded. Mainly two methods have been used to reduce the chances that participants were aware of the presented stimuli: subliminal priming and subliminal presentation of fear conditioned stimuli. From this literature a lack of agreement on various methodological aspects was apparent as well as a large variety of outcome measures. Nevertheless, the findings seem to indicate that negative affective stimuli in subliminal priming studies increase systolic blood pressure (SBP) relative to the control stimuli. In the fear conditioning studies, fear conditioned stimuli that were very briefly presented increased skin conductance response (SCR) amplitude. Insufficient data were available for other physiological outcomes. Notably, none were performed with hormonal or immunological outcomes. Taken together, based on this overview I cannot conclude with certainty that unconscious stress, operationalized as negative affective stimuli presented outside of awareness, negatively influences health-relevant parameters, such as blood pressure (BP) and heart rate variability (HRV; 95). Not only do the findings call for more studies using these parameters, it also stresses the need for

more consensus in the field regarding adequate subliminal presentation, awareness checks, consistency in data reporting and interpretation, and execution of replication studies.

The three subsequent chapters described studies in which the unconscious stress hypothesis was tested using both methods to manipulate awareness of stimuli mentioned above: subliminal priming and subliminal presentation of fear conditioned stimuli.

In **Chapter 3**, subliminal priming was used to test the unconscious stress hypothesis. Healthy participants were presented either threatening or neutral words (the primes), while performing a categorization task. Although mean arterial pressure (MAP) and total peripheral resistance (TPR) were higher and heart rate variability (HRV) was lower in the threat condition as expected, statistical significance was only obtained for TPR. Interestingly, TPR is believed to be associated with perceived threat (99,100). In addition to this main finding, moderating effects of trait worry and baseline HRV, which are common predictors of adverse health outcomes, were not related to the CV reactivity to the different primes. Furthermore, using the Implicit Positive and Negative Affect Test (IPANAT, 84) as implicit measure of affect after the priming task, a small and statistically marginal significant positive relationship was found between implicit positive affect (IPA) and mean arterial pressure (MAP), but overall implicit affect was not related to CV reactivity to the primes. Thus, unconscious stress, operationalized as threat words presented outside of awareness, can elicit an increase in TPR, which can be interpreted as a physiological state reflecting perceived threat. However, the priming effects appear to have been too weak to sort effects on other affective and CV parameters.

One of the problems in the field is the lack of replication studies to validate previous findings (see Chapter 2). Therefore, **Chapter 4** describes a replication of studies of Hull et al. (2002, 62) and (partially) of Garfinkel et al. (2016, 61), that showed that repetitive subliminal presentation of the prime word 'angry', increased blood pressure (BP) and heart rate (HR) in comparison with the prime 'relax'. In the study in Chapter 4 none of these differential changes in BP or HR were replicated. Furthermore, in contrast to expectations, higher implicit negative affect (INA) was related to lower SBP and diastolic blood pressure (DBP) during the priming task. In line with the expectations, lower IPA was related to a higher TPR during priming as well as during the recovery phase. Thus, the primes did not seem to affect the physiological state, but affect measured with an implicit measure did relate to CV activity. This indicates that although the subliminal presentation of primes may not have been successful, implicit measures may provide additional information in the relationship between psychological stress and CV activity.

In **Chapter 5**, fear conditioning was used to create a stressor that was equal across participants. The stressor was then presented subliminally. Neutral stimuli

were paired with a shock (CS+) set at an intensity that was barely tolerable for the participants. Other neutral stimuli were not paired with the shock (CS-). The CS+ and CS- were presented above and below the threshold of awareness. In this study, skin conductance was also included as a physiological measure since Chapter 2 indicated that this is a widely used measure in the field, and would therefore provide a good comparison with previous studies. SCR magnitude was larger in response to the CS+, compared to the CS-, also when CS+ stimuli were presented subliminally. However, no such effects were found for the CV variables. Thus, only partial evidence for the unconscious stress hypothesis was obtained, which may indicate that fear conditioned stimuli outside of awareness may affect the physiological state, but not necessarily in a way that is detrimental to health.

A different approach to unconscious processes was to assess stress-related processes with so called *implicit measures* and relate these to CV activity during and after a laboratory stressor. In **Chapter 6**, psychological stress was induced using a mathematical task with and without anger harassment. The IPANAT was used as an implicit measure to capture implicit positive and negative affect, while a self-report measure of affect was used to capture explicit or 'conscious' assessment. Both were administered during the recovery phase. The addition of harassment to the mathematical task did not yield additional CV reactivity or recovery. Importantly, higher INA was related to higher SBP, and lower TPR and HRV during the task and to slower recovery of DBP, while lower IPA was related to slower recovery of SBP and DBP. Thus, the relationship of the IPANAT subscales with CV recovery after a stressor at least seems to indicate that CV activity might partially be explained by implicit affective processes, as predicted by the unconscious stress hypothesis.

In **Chapter 7**, an anger recall procedure was used to induce psychological stress, as a different laboratory stressor, which was compared to 'happy recall' as a control condition. This time, an affective Lexical Decision-making Task (LDT; 89) was used as implicit measure of unconscious stress, indicated by cognitive activation of information and expressed as automatic vigilance (81). Again, no differences in CV reactivity or recovery were found between the conditions, but a higher automatic vigilance index for negative information (AVI-N) was related to a higher DBP during the recovery and a lower automatic vigilance index for positive information (AVI-P) was related to slower recovery of SBP, HR, and TPR. Importantly, self-reported affect was not related to CV reactivity or recovery.

An overview of the main findings in this thesis is presented in Table 1. In short, we could not replicate the increased SBP found by the systematic review in Chapter 2 using subliminal presentation of negative affective stimuli versus control stimuli (Chapters 3, 4, & 5). However, we did find some support with respect to the TPR effects found in Chapter 2 using general threat words versus neutral words (Chapter 3). Furthermore, higher INA was related to decreased SBP and DBP in response to the

TABLE 1 Summary of the main findings presented in this thesis

Method	Unconscious stress operationalization	Main findings			
		CV activity		*Other outcomes*	
Systematic review (Chapter 2)	Negative affective stimuli versus nonnegative affective stimuli presented subliminally	SBP (12) ↑ DBP (10) ↔ HR (18) ↔ PEP (8) ↔ HRV (6) ↔	CO (1) ↑ VC (1) ↔ TPR (1) ↑ RR (1) ↔	SCR amplitude (27) ↑ SCR magnitude (9) ↔ SCR rise time (1) ↑ SCR frequency (2) ↔ SCR latency (1) ↔ GSR (4) ↑ SCL (4) ↑	Corrugator supercilii (20) ↔ Orbicularis oculi muscles (7) ↑ Zygomatic major (14) ↔ General EMG activity (2) ↔
Subliminal priming (Chapter 3)	Threat-related versus neutral words presented subliminally	MAP ↔ RMSSD ↔ TPR ↑		↑ INA: MAP ↔, RMSSD ↔, TPR ↔ ↑ IPA: MAP ↔, RMSSD ↔, TPR ↔	
Subliminal priming (Chapter 4)	'Angry' [woedend] versus 'relax' [rustig] presented repeatedly and subliminally	SBP ↔ DBP ↔ HR ↔		↓ INA: SBP ↑ , DBP ↑ , HR ↔, TPR ↔ ↓ IPA: SBP ↔, DBP ↔, HR ↔, TPR ↑	
Fear conditioning (Chapter 5)	Fear conditioned images (CS+) versus neutral images (CS-) presented subliminally and supraliminally	SBP ↔ DBP ↔ HR ↔		SC magnitude ↑	
Mathematics and anger harassment (Chapter 6)	IPANAT as implicit measure	*Reactivity*: SBP ↑ DBP ↑ HR ↔ RMSSD ↔ TPR ↓	*Recovery*: SBP ↔ DBP ↔ HR ↔ RMSSD ↔ TPR ↔	*Reactivity*: ↑ INA: SBP ↑ , DBP ↔, HR ↔, RMSSD ↓ , TPR ↓ ↑ IPA: SBP ↔, DBP ↔, HR ↔, RMSSD ↔, TPR ↔ *Recovery*: ↑ INA: SBP ↔, DBP ↑, HR ↔, RMSSD ↔, TPR ↔ ↓ IPA: SBP ↑ , DBP ↑, HR ↔, RMSSD ↔, TPR ↔	
Emotional recall (Chapter 7)	LDT as implicit measure after an anger or happy recall procedure	*Reactivity*: SBP ↑ DBP ↑ HR ↔ RMSSD ↔ TPR ↓	*Recovery*: SBP ↔ DBP ↔ HR ↔ RMSSD ↔ TPR ↔	*Reactivity*: ↑ AVI-N: SBP ↔, DBP ↑ , HR ↔, RMSSD ↔, TPR ↔ ↑ AVI-P: SBP ↔, DBP ↔, HR ↔, RMSSD ↔, TPR ↔ *Recovery*: ↑ AVI-N: SBP ↔, DBP ↔, HR ↔, RMSSD ↔, TPR ↔ ↑ AVI-P: SBP ↔, DBP ↓, HR ↓ , RMSSD ↔, TPR ↑	

Note. ↑ = Increase, ↓ = Decrease, ↔ = No change. For Chapter 2 the number of studies on which the conclusion is based is indicated between brackets. Regarding recovery, higher values indicate slower recovery (Chapter 6) and lower values indicate faster recovery (Chapter 7). *Abbreviations*: CV = Cardiovascular, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, PEP = Pre-ejection period, HRV = Heart rate variability, CO = Cardiac output, VC = Ventricular contractions, TPR = Total peripheral resistance, RR = Respiration rate, SCR = Skin conductance response, GSR = Galvanic skin response, SCL = Skin conductance level, EMG = Electromyographical, MAP = Mean arterial pressure, RMSSD = Root mean squared successive differences, INA = Implicit negative affect, IPA = Implicit positive affect, CS = Conditional stimulus, IPANAT = Implicit Positive and Negative Affect Test, LDT = Lexical Decision-making Task, AVI-N = Automatic vigilance index for negative words, AVI-P = Automatic vigilance index for positive words

word 'angry' versus 'relax' (Chapter 4). In two studies with explicit stress-inducing situations, high INA was related to higher SBP and lower HRV and TPR during the stressor, but during recovery from the stressor low IPA was related to slower recovery of SBP and DBP (Chapter 6). Furthermore, high AVI-N was related to increased DBP during the stressor, but high AVI-P was related to faster recovery of DBP and TPR.\

Implications for the unconscious stress hypothesis

The findings provide tentative support for the unconscious stress hypothesis. Across studies, we found that subliminally presented stress-related stimuli increased TPR, but not SBP, DBP, and HR, and we found no decrease of HRV. Additionally, in the fear conditioning study in particular we found increased SC magnitude to the stress-related stimuli above and below the threshold of awareness. Furthermore, the implicit measures of psychological stress were related to CV activity during a stressor (Chapter 6), albeit sometimes in the opposite direction of what was expected, and after (Chapters 6 and 7) a stressor, where the explicit measure was not related to the CV activity. The unconscious stress hypothesis states that stress-related cognition may occur outside of awareness by which it negatively affects the physiological state, in addition to what is within the realm of awareness (26,27). Based on the findings, it seems evident that what is outside of awareness may influence the physiological state, but not on all health-relevant parameters, and not for all stressful stimuli and stressors used. Consequently, the unconscious stress hypothesis is only partially supported.

The crucial issue in addressing unconscious stress has been its operationalization. In line with the definition of psychological stress (16), we focused on the activation of affective representations of a stressor. In all studies we intended to activate or measure these representations and expected concurrent physiological responses. However, the different operationalizations of unconscious stress in the studies were inconsistently related to physiological changes. An explanation may be that we erroneously assumed that the various stressful stimuli and situations used would all have physiological effects. Possibly, the stimuli used were either too general or too mild. In contrast, previous studies that used specific stimuli related to the specific samples (e.g., negative stereotypes of ageing in elderly people; 63, social ties with real friends/acquaintances; 199) found clear CV effects.

The anger primes of Chapter 4 and the fear conditioned stimuli in Chapter 5 may have been too mild. As we also argue in the respective chapter, perhaps the word 'angry', at least the Dutch translation 'woedend', may not be sufficiently intense to influence CV activity. This may also hold for the fear conditioned stimuli: although the shock was perceived as uncomfortable, participants indicated that the intensity could have been higher in hindsight, which has been suggested to be a frequent methodological issue (269). In addition to the comparison of our findings with the replication of Garfinkel et al. (2016, 61) and Hull et al. (2002, 62) other factors mentioned

in the systematic review in Chapter 2, such as the type of stimuli, may be of influence as well. The research group of Gendolla (e.g., 132, 144, 163) has repeatedly found effects of subliminally presenting depictions of facial expressions on CV activity. However, they measured CV activity during a (demanding) math task and focused on the effects of task difficulty in relation to the primes. Thus, perhaps it is another factor, for example adding additional cognitive demands, that intensifies the CV response patterns to subliminal stimuli. Still, presenting fearful faces subliminally has been shown to elicit amygdala activation associated with detection of salient stimuli (72). One could then argue that subliminal processing of emotional expressions is different (i.e., has stronger effects) from that of other images or words, as used in the current dissertation. Nevertheless, another study that reported changes in CV activity used religious images (172). The conclusion then seems inevitable that this matter needs further and more systematic research. All in all, it is still possible that in real life, outside the laboratory, stress-related information above or below threshold of awareness elicits much stronger affective responses, and that this information has considerable physiological effects. It might be difficult to model these effects in the laboratory, except when the stress-related stimuli used are individualized and meaningful.

Additionally, we assumed that affective representations of the stressor outside of awareness would be induced by subliminal presentation and measured indirectly with the implicit measures. However, although affective changes processes outside of awareness may exist, but we can never be certain that a change in affectivity occurred nor that induction and assessment occurs without conscious processing by the participants, not even when changes on the IPANAT and LDT were observed (144,194,195). Thus, the partial support provided here is fuel for more questions regarding unconscious processes in this line of research. The idea that psychological stress may also be unconscious is therefore not dismissed in this thesis, rather it shows that the existence of the phenomenon is probable and deserves further exploration. In other words, based on the current findings but also the literature, I think it is fair to state that we have only scratched the surface on the continuum of consciousness in cardiovascular stress research.

Theoretical considerations beyond unconscious stress

The expedition undertaken in this thesis ran into various alternative options and point of views that I feel should be explicated in this discussion if one is to build upon the knowledge gathered. First of all, from the systematic review (Chapter 2) it became apparent that there are various theoretical viewpoints on unconscious processes and physiological activation that influence study designs and conclusions, despite using similar methodologies. From these different viewpoints two are relevant in terms of the current findings. The "preparedness theory" (186,268) states that fear

conditioning of subliminal findings can only be successful when the stimuli relate to evolution-based aversive images, such as snakes. However, I have argued that the fear conditioning itself, that is, combining a neutral image with an aversive stimulus, creates a stimulus that instigates threat in the individual, which then generates a physiological response in Chapter 5. Although we did not find an effect of the fear conditioning procedure on the CV variables, skin conductance measures did show this effect (Chapter 5), both supraliminally and subliminally. Thus, the preparedness theory did not hold in the current set-up.

Furthermore, it has been suggested that implicit affect can facilitate coping with challenges in the so-called *implicit-affect-primes-effort model* (IAPE model; e.g., 331-333). In other words, subliminal priming of the affective state can influence whether a task is considered easy (i.e., in case of activated happiness and anger) or difficult (i.e., in case of activated sadness and fear). The former case (i.e., easy) would be characterized by mainly higher cardiac responses (PEP and HR) to a difficult task, while the latter (i.e., difficult) would be characterized by more vascular responses (TPR). In the studies where we presented subliminal stimuli, we used the word 'angry' (which would induce perceived task easiness and dominantly PEP and HR), threat-related words (which would induce perceived difficulty and dominantly TPR), and fear conditioned images (also perceived difficulty). Notably, we used a simple categorization task (Chapters 3 and 4) and no task (Chapter 5) rather than a highly demanding cognitive task that is commonly used in studies testing the IAPE model. However, we found no effects on HR, but PEP was not used as an outcome measure. Moreover, only vascular activity (TPR) appears to have been affected, and only with threatening stimuli. So, regardless of the specific valence of the stimuli, there was no evidence for an effect of subliminal primes on effort-related cardiac responses.

Another related theoretical paradigm, that was not addressed in the systematic review, is the idea of the existence of a core affect, which is a neuropsychobiological state described in terms of the dimension pleasure-displeasure and that of activation-deactivation (310). It challenges the traditional 'basic emotions' view (e.g., 334) on emotions that suggests that an event leads to an emotion, which then triggers feelings, behavioral, and physiological responses (for a review see 335). In core affect, physiological responses are part of the concept itself that prepare the individual for action. In contrast, self-reported 'feelings' are considered to be an affective quality assigned to a specific situation or object, which does not necessarily relate to the core affect. The hypothesis of core affect seems theoretically related to the unconscious stress hypothesis, which also describes a nonspecific change in (negative) affectivity (26,27). Importantly, the findings from the current thesis confirm that processes beyond self-report are related to physiological changes, but also that the affective processes are likely to operate on a two-dimensional plane of pleasure/displeasure and activation/deactivation (310) rather than in specific categories of emotion, such

as fear or anger, referring to the findings regarding positive affectivity. Therefore, the role of core affect might be an interesting line of research in stress and health, encompassing emotion-theory and processes beyond awareness.

Contributions to stress research

Apart from offering partial support for the notion that stress-related physiological activity is to some extent caused by unconscious stress, that is, the unconscious stress hypothesis, this thesis potentially contributes to stress research in several additional ways.

First, and perhaps somewhat surprising, is the finding that TPR, and not the other CV variables, appeared to be most sensitive to unconscious stress. Specifically, although the SBP, DBP, and HR may not have been affected by subliminal priming and subliminally presented fear conditioned images, TPR was changed. It increased in response to subliminally presented threat-related words (as expected; Chapter 3). Furthermore, during a mental challenge and emotional task it decreased (not as expected; Chapters 6 and 7). Additionally, a higher TPR was found to be related to lower IPA during and after a priming task (as expected; Chapter 4) and to lower INA during a difficult mental task (not as expected; Chapter 6) as measured with the IPANAT, but also to a higher AVI-P after emotional recall as measured with the LDT (not as expected; Chapter 7). A vascular response pattern to psychological stress, as contrasted with a cardiac response pattern, may be particularly detrimental for health as it has been related to hypertension and hypertrophy (336-338) which may have severe consequences for health (97,98,339). Furthermore, TPR has been found to increase in response to worrying (340). Moreover, TPR has been related to psychological stress and is believed to be specifically related to threat appraisal (99,100,172,341), that is, when a situation is appraised as threatening, TPR increases. Additionally, the Cognitive Activation Theory of Stress (CATS; 16) describes that only after a stimulus is appraised as threatening, it is followed by a nonspecific stress response and cognitive processing. In other words, the observed changes in TPR in response to the stress-related stimuli are in line with the psychobiosocial model of Blascovich (99) and our definition of psychological stress that is based on the CATS. Moreover, the findings regarding TPR may be a key element in the relationship explaining the relationship between psychological stress and adverse CV health outcomes. Based on the current findings, it could be argued that TPR is indicative of psychological stress, regardless of the level of awareness at which the stressor is processed. Thus, in light of the current findings, it appears that TPR may be a sensitive outcome measure that responds to stress inductions even outside of awareness.

Another implication of the findings is the evident additional explanatory value of the implicit measures in stress research. When implicit measures were taken into account, they were related to the physiological changes, over and above the self-report

measures of psychological stress. Moreover, self-reported affectivity was not related to the CV activity. These findings not only call for the application, further development, and testing of additional *implicit* measures, but in general may also explain why the causal relationship between CV health and psychological stress remains unresolved. This supports the unconscious stress hypothesis and stipulates the additional value of these measures in explaining the relationship between psychological processes and health.

Finally, two unintended findings in this thesis challenge the use of some standard laboratory stressors without proper control manipulations. We found that anger harassment in addition to a neutral mathematical task and that anger recall versus happy recall did not result in differentiating CV changes. In stress research, there are several methods to induce stress that are widely used and accepted within the field, including anger harassment and anger recall procedures (92). However, these tasks are mostly used in a battery of multiple tasks and lack adequate control groups. Most studies merely compare healthy versus non-healthy participants in CV reactivity on these tasks (92,342). In contrast, in the current thesis we aimed to use control groups in our designs to ensure that the findings would be attributable to the negative affective component rather than other cognitive processes such as calculating or emotional recall. Surprisingly, the mental arithmetic and anger harassment elicited similar CV responses, as did the anger and happiness recall. This seems to call into question the role of angry affect as such, to elicit these physiological effects. Practically, these findings imply that certain widely used stress manipulations to induce CV responses in the literature have not been sufficiently controlled for. In other words, this is a setback in our understanding of the mechanisms underlying psychological stress and CV activity, at least in healthy individuals, which should encourage researchers to formulate statements on this relationship in non-healthy samples with conceptual caution.

Strengths and limitations

A strength of the current thesis is the overarching approach undertaken to address the unconscious stress hypothesis and to expand knowledge on the relationship between processes outside of awareness with negative affective associations and peripheral physiological responses. I have used several experimental designs that applied to the theoretical notions described in Chapter 1. The use of these various methodologies based on commonly executed psychological experiments, provides a new overview of the important issues regarding the different methods and findings in light of their relevance to the unconscious stress hypothesis. The combination of experiments provides a fruitful base for further research on this topic.

A second strength of this thesis is the careful localization and summary of earlier similar studies and their results. The new method of finding and systematically testing

all possible keywords in a single, changing keyword profile, enabled us, and will enable others, to point out much more precisely the state of the art, or 'what we know' and what we 'do not yet know'. It also indicates that building upon literature to which one is familiar is not sufficient and a much more thorough search following the proposed method is needed to find all relevant literature (Chapter 2). Moreover, these studies were evaluated on the quality of the reported information in terms of risks for bias. This is a common requirement in for example the medical sciences exemplified by the Risk of Bias Tool by the Cochrane collaboration (343), but such evaluations of quality have not been previously applied to psychological experimental research. The quality ratings in Chapter 2 disclosed the lack of replication studies and limitations in reporting and peer-review. This unnecessarily frustrates the progress of science as it could be easily addressed by for example pre-registration and open access publication of datasets. In line with our own recommendations, but limited by financial resources, we have published one chapter open access (Chapter 6) and the data from the respective papers of all the chapters have been made accessible online. By providing a systematic method for literature search, a tool to quantify the quality of psychological experiments, and making the data of this thesis available online (see section 'Publications'), I have strived to contribute to the academic landscape beyond the scope of this thesis.

A third strength of this thesis is that we aimed to conceptually replicate two studies (61,62) of which the outcomes provided substantial support for the unconscious stress hypothesis (Chapter 4), namely an increased CV response to the word 'angry' versus the word 'relax'. However, we were not able to obtain the same results. Failures to replicate may be attributed to differences between the current and the original study (e.g., 258), but effects of subliminal priming are rarely replicated (60), which seems to be applicable in this study. Moreover, this nonreplication once again shows the importance of verifying the results of previous studies before drawing firm conclusions based on the data. Having stated this, I would encourage replication of the findings in this thesis and the conclusions as I have stated them since they should also be sufficiently verified (or falsified).

Despite these strengths, the work also has several limitations that should be addressed. First, the thesis does not contain a systematic review and/or meta-analysis on the relationship between implicit measures and peripheral physiological responses. Therefore, although we have tried to be sufficiently comprehensive in the respective chapters, we cannot state with certainty that all available information has been used to determine the study designs and weigh the findings regarding the role of implicit measures in stress research. A second limitation is the lack of a baseline measurement of the implicit and self-report measures in Chapters 6 and 7, which implies that for these state measures we could not sufficiently check for pre-existing values or trait aspects of the measured constructs. However, we chose this design to prevent

carry-over effects of the measurement onto the manipulation, CV activity, and the measurement of interest afterwards. Finally, we have attempted in the studies with subliminal presentations to ensure that participants were unaware of the stimuli and used awareness checks to verify the success of these attempts (121,122,256). However, neither subjective or objective measures of awareness will be fully able to capture what the participants actually saw (194-196).

With respect to the implicit measures, there are several more options to consider (e.g., 74,81). One option that we have considered was the Morphing Faces Task (MFT, 327), as described in Chapter 7, which we thought would represent a tendency to more readily detect faces displaying (negative) affectivity after a stressor. However, due to high correlations amongst the different expressions, the test could not be considered reliable and could not be used further to address the research question. Another task, the Approach Avoidance Task (344-347), is particularly interesting. In this task, participants have to avoid or approach a stimulus on the screen based on an irrelevant feature (e.g., color) with a joystick which lead to a decrease or increase in size of the stimulus. The stimuli are usually images with a relevant content of two categories, such as angry versus happy faces (e.g., 348), insects versus noninsects (349), or alcoholic beverages versus soft drinks (350). Although the AAT seems a promising implicit measure, in the application to stress research it has some disadvantages. Similar to the IAT, and as discussed in Chapter 1, it can only be used for two categories which is very limiting considering the nonspecific nature of psychological stress (CATS; 16,74). Furthermore, when looking at the outcome measure, it indicates a tendency to either avoid or to approach. However, in a stressful situation one may be inclined to do both, that is, one may want to approach the stressor and confront it, or one may want to avoid the stressor (e.g., 351,352), and both may be associated with an unconscious stress experience. This would complicate interpretation of the findings in this context. All in all, we have not further pursued the ideas around the AAT.

Future directions

Based on the current findings and relating literature, we see several future directions of studies on unconscious processes in stress research in addition to those already described in the previous chapters. There are several other methods that we have not applied, but that I think should be considered.

First, an idea that is not new, but simply has not been addressed here, is the role of personality traits or other more or less stable characteristics that relate to the inability to (verbally) address one's mental state, such as alexithymia or levels of emotional awareness (28). People with alexithymia may to some degree be less equipped to identify, express, and regulating their emotions, but also an increased self-reporting of negative affectivity (353-355) which has been related to all-cause mortality (356), and CV disease (357-359). This relationship has been explained by

autonomic dysregulation or adverse coping behavior originating from the characteristics of alexithymia (360,361). Furthermore, emotional awareness can be described in several levels that indicate the degree to which one is able to recognize and describe one's own emotions and that of others (362). Lower levels of emotional awareness have been related to for example hypertension (28). These constructs are two examples of explanations why some develop somatic diseases based on dysregulation of emotion processing. Other examples of this restricted reporting of emotions, which should not be confused with the absence of experiencing emotions (e.g., 353), are Type D personality, neuroticism, and defensiveness (as described in 28). Future studies could include these characteristics to relate to stress reactivity and recovery and development of (CV) disease over time in addition to self-report and implicit measures of mental stress.

Second, in this thesis we have mainly focused on response-based measures of psychological stress, self-report and implicit, to explain CV activity. The unconscious stress hypothesis is partially based on the absence of sufficient explanatory value of the self-report measures (25-27). However, recording other physiological or behavioral modalities that inherently do not require any inquiry from participants may further clarify CV responses to psychological stress. One can think of the use of pupil dilation, which has been shown to provide information about mental load (363), or eye movements, which have been found useful in distinguishing fearful from nonfearful subjects (364). Another example is the use of EMG measurements as described in Chapter 2. Facial muscle activity has been studied as a form of nonverbal behavior and emotional expression or experience (365,366), which could provide an additional source of information regarding the affective state and its relationship with health (367). Moreover, several methods of assessing and categorizing facial emotional expressions have been developed (e.g., 368-370) and e-health applications are currently evaluated (e.g., 371).

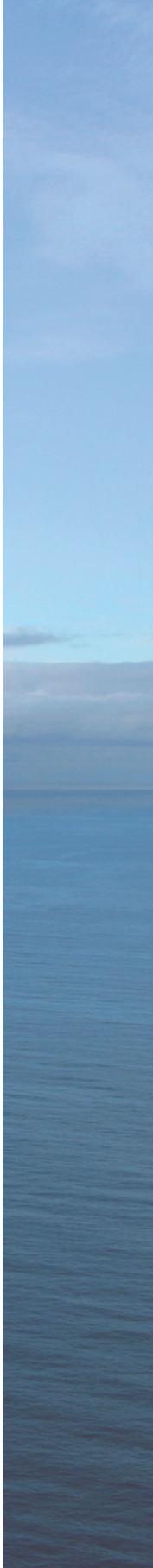
Third, I believe that the clarification of the relationship between psychological stress and CV health is hindered by a suboptimal use of the collected data in psychosomatic medicine and psychophysiological research. This could be overcome through the implementation of advances in statistical analytical techniques. One can think of using machine learning to predict participants' outcomes (e.g., 372), and quantum mechanical techniques to understand the sensation-perception dynamics (373), but also of the shift away from the interpretation of *p* values (e.g., 374-376). Perhaps further progress in psychological science can be achieved by building upon (and re-analyzing) existing data and taking this knowledge into the 21st century.

Final conclusions

Taking together all the findings and literature discussed here, I have found tentative verification of the unconscious stress hypothesis. It has also become clear that, from

what has turned out to be a first expedition into the continuum of consciousness in cardiovascular stress research, there is a remaining abundance of options available to assess and influence processes outside of awareness that can be applied in the context of psychological stress and health.

"There is great need to spell out explicitly the assumed characteristics of the unconscious and to search for explanations of so called unconscious phenomena in terms of more commonplace psychological variables. To do so may destroy the titillating mystery that the unconscious seems to hold but then that is the business of science."
Eriksen (1960, p.120)



Nederlandse samenvatting

Psychologische stress heeft een negatieve invloed op de gezondheid en kan bijdragen aan het ontstaan of verergeren van lichamelijke ziekten zoals hart- en vaatziekten (2-7). Deze negatieve relatie wordt toegeschreven aan chronische stressoren, bijvoorbeeld werkstress, en verminderd fysiek herstel van stressoren (10-16). Diverse studies lijken aan te tonen dat dit samenhangt met de continue aanwezigheid van stress-gerelateerde cognities, zoals bij piekeren, die de fysieke reacties op stressoren in stand houden alsof de laatstgenoemden nog daadwerkelijk aanwezig zijn (17-21). Deze aanhoudende fysieke activiteit wordt echter niet volledig verklaard door gerapporteerde stress-gerelateerde cognities (22-25). Het zou kunnen zijn dat deze cognities ook bestaan zonder dat we ons daar bewust van zijn en desondanks bijdragen aan de langdurige fysiologische activiteit die uiteindelijk kan leiden tot ziekte. Dit noemen we onbewuste stress (26-28).

In deze thesis hebben we onderzocht of onbewuste stress samenhangt met fysieke activiteit om de relatie tussen psychologische stress en lichamelijke ziekten te verhelderen. In een serie van experimenten hebben we psychologische stress geïnduceerd en de fysieke activiteit gemeten in gezonde proefpersonen. Hierbij zijn er diverse methoden zijn toegepast om onbewuste stress te adresseren: door stress-gerelateerde stimuli onder de waarnemingsgrens aan te bieden (subliminaal) of door de ervaring van psychologische stress te meten zonder dat direct aan de proefpersonen te vragen, aan de hand van een zogeheten impliciete maat. Bovendien hebben we aan de hand van een systematisch literatuuroverzicht gekeken wat er tot nu toe bekend was over het effect van subliminaal aangeboden stress-gerelateerde stimuli op fysiologische activiteit. Aan de hand van deze studies hebben we getracht aan te tonen dat onbewuste stress-gerelateerde cognities samenhangen met fysiologische activiteit die doorgaans, indien het langdurig aanhoudt, samenhangt met een verslechtering van de gezondheid.

Belangrijkste bevindingen

In **Hoofdstuk 2** hebben we de uitkomsten van 65 experimentele studies verzameld, waarin het effect op perifere fysiologische activiteit van negatief affectieve stimuli werden afgezet tegen de effecten van controle stimuli wanneer beiden subliminaal werden aangeboden. Er kwamen twee methoden naar voren: subliminale priming, waarbij de stimuli een bepaalde intrinsieke emotionele waarde (valentie) hebben, en het subliminaal aanbieden van met angst geconditioneerde stimuli door zogenaamde vrees-conditionering toe te passen. Het bleek dat negatieve affectieve stimuli bij subliminale priming met name systolische bloeddruk verhogen in vergelijking met de controle stimuli en dat na vrees-conditionering met name de huidgeleiding-amplitude hoger was in vergelijking met de controle stimuli. Uit deze analyse bleek echter ook dat er weinig consensus bestaat over belangrijke methodologische

aspecten, zoals het controleren van daadwerkelijke onbewuste verwerking van de stimuli en de exacte duur van een subliminaal aangeboden stimulus, ondanks dat de studies op conceptueel niveau sterk vergelijkbaar waren. Bovendien zijn er zeer veel verschillende uitkomstmaten gebruikt waardoor vergelijking van de resultaten maar beperkt mogelijk is. Hoewel de resultaten consistent zijn met de hypothese dat onbewuste stress fysiologische consequenties heeft, kunnen we op basis van deze studie dus nog geen definitieve stelling nemen over het effect van subliminale stress-gerelateerde stimuli op de fysiologische activiteit. Los daarvan biedt de studie wel een overzicht van de uitdagingen in het onderzoeksgebied die zullen moeten worden aangegaan om op constructieve wijze bij te dragen aan de kennis van het effect van subliminale stimuli op (perifere) fysiologische uitkomstmaten.

In **Hoofdstuk 3** hebben we subliminale priming toegepast waarbij er bedreigende woorden of neutrale woorden (de primes) subliminaal werden getoond aan de proefpersonen terwijl ze een andere irrelevante taak uitvoerden. Zoals verwacht waren de gemiddelde arteriële druk en totale perifere weerstand hoger en de hartslagvariabiliteit lager bij het tonen van bedreigende woorden, maar alleen voor totale perifere weerstand in het vaatstelsel was dit verschil met de neutrale woorden statistisch significant. Een onafhankelijke verandering van totale perifere weerstand is al eerder in verband gebracht met waargenomen dreiging (99,100), piekeren (340) en verhoogde kans op hart- en vaatziekten (97,98,336,399). De resultaten sluiten aan bij onze hypothese dat onbewuste stress een fysiologisch effect kan hebben. De effecten bleken niet samen te hangen met een neiging om te piekeren en een lage baseline hartslagvariabiliteit. Daarnaast was er geen effect van impliciet gemeten negatieve en positieve affectiviteit met de Implicit Positive and Negative Affect Test (IPANAT, 252-254) op de fysiologische activiteit. Concluderend, in deze studie vonden we dat wanneer bedreigende woorden onder de waarnemingsgrens worden getoond dit de totale perifere weerstand kan beïnvloeden wat op termijn negatieve effecten op de gezondheid kan hebben.

In **Hoofdstuk 4** hebben we de resultaten van twee studies (61,62) die het bestaan van onbewuste stress lijken te bevestigen geprobeerd te repliceren. Ook hier gaat het om een subliminale priming taak, waarbij we echter het woord ‘woedend’ of ‘ontspan’ 100 keer hebben gepresenteerd. In de eerdere studies leidde deze manier van aanbieden van het woord ‘angry’ versus ‘relax’ tot een verhoogde bloeddruk en hartslag, maar wij hebben dat niet kunnen repliceren. Aanvullend op deze bevindingen vonden we onverwachts wel dat hogere negatieve impliciete affectiviteit, gemeten met de IPANAT, samenhing met een lage bloeddruk tijdens de taak en een lagere positieve impliciete affectiviteit met een hogere totale perifere weerstand tijdens én na de taak. Kortom, het effect van de stress-gerelateerde prime op de fysiologische staat werd niet gevonden, maar impliciet gemeten affectiviteit verklaarde wel verschillen in cardiovasculaire activiteit.

In **Hoofdstuk 5** gebruikten we een andere manier om onbewuste stress te induceren: vrees-conditionering. Hierbij hebben we neutrale plaatjes gecombineerd met een schokje (CS+) waardoor zij als stress-gerelateerde stimuli fungeerden. Andere neutrale plaatjes werden niet gepaard met een schokje (CS-). De CS+ en CS- werden subliminaal en supraliminaal (boven de waarnemingsgrens) getoond. Naast bloeddruk en hartslag keken we in deze studie ook naar huidgeleiding, omdat dit een veel gebruikte uitkomstmaat is in vrees-conditioneringsstudies en we zo het succes van de manipulatie konden evalueren. Het bleek dat huidgeleiding-magnitude groter was na de CS+ dan na de CS-, zowel supraliminaal als subliminaal. Op de cardiovasculaire uitkomstmaten vonden we geen verschillen. Deze studie heeft dus ten dele bewijs gevonden voor onbewuste stress. Mogelijk betekenen deze resultaten dat milde stressoren buiten bewustzijn wel de fysieke staat kunnen veranderen, maar niet direct een negatief effect hebben op factoren die van invloed kunnen zijn op de gezondheid.

Een geheel andere aanpak wordt besproken in de volgende twee hoofdstukken. In **Hoofdstuk 6** werd een rekentaak gebruikt om psychologische stress op te wekken, waarbij er in de ene groep negatieve feedback werd geuit op de prestatie en in de andere groep geen feedback werd gegeven. Na deze taak werd de affectiviteit gemeten met een impliciete maat (de IPANAT) en een expliciete taak (visuele analoge test). Er was geen aanvullend effect van de negatieve feedback op de uitkomstmaten. Echter, een hoog negatieve impliciete affectiviteit hing samen met een hogere systolische bloeddruk en lagere totale perifere weerstand en hartslagvariabiliteit tijdens de taak. Een lagere positieve impliciete affectiviteit hing samen met een langzamer herstel van de diastolische bloeddruk na de taak. De expliciete taak hing niet samen met de uitkomstmaten. Het gebruik van de impliciete maten geeft dus aanvullende informatie ten opzichte van expliciete maten. Dit bevestigt dat er psychologische stress kan zijn buiten wat normaliter gerapporteerd wordt, en dus het bestaan van onbewuste stress, en suggereert dat die samenhangt met gezondheid-gerelateerde uitkomsten.

In **Hoofdstuk 7** werd als stressor een taak uitgevoerd waarbij proefpersonen over een gebeurtenis moesten vertellen die hen boos dan wel blij hadden gemaakt. Ook na deze taak werd de affectiviteit gemeten met een expliciete maat (visuele analoge taak). Als impliciete maat werd een lexicale beslis-taak (89) gebruikt die de cognitieve activatie van negatieve en positieve informatie weergeeft (81). Ook in deze studie vonden we geen verschil tussen de groepen. Tegelijkertijd vonden we wel een relatie tussen een verhoogde activatie van negatieve informatie en een hogere diastolische bloeddruk tijdens herstel van de taak, en een relatie tussen een lagere activatie van positieve informatie en een langzamer herstel van systolische bloeddruk, hartslag en totale perifere weerstand. Bovendien hing de expliciete affect maat wederom niet samen met de fysiologische activiteit. De resultaten van deze studie komen overeen

met de voorgaande studie en geven aan dat onbewuste processen een rol spelen in de psychofysiologische stressrespons.

Samengevat, onze studies in Hoofdstukken 3, 4 en 5 vonden we geen verhoging van systolische bloeddruk in reactie op subliminale stress-gerelateerde stimuli die we vanuit de literatuur hadden verwacht (Hoofdstuk 2). Wel vonden we dat totale perifere weerstand hoger was na subliminale bedreigende woorden vergeleken met subliminale neutrale woorden (Hoofdstuk 3). Daarnaast waren de negatieve én positieve subschalen van de impliciete maten gerelateerd aan cardiovasculaire activiteit (Hoofdstukken 4, 6 en 7), waarbij het met name opviel dat lagere waarden op de positieve subschalen gerelateerd waren aan fysiologische activiteit die als schadelijk voor de gezondheid zou kunnen worden gezien. Zoals uiteengezet wordt in Hoofdstuk 8 is de algehele conclusie die op basis van dit proefschrift gesteld kan worden dan ook dat er aanwijzingen lijken te zijn dat onbewuste processen een rol spelen bij psychofysiologische stress, maar dat er meer en grondig onderzoek nodig is, aan de hand van de in deze thesis gebruikte methoden, om een meer definitief perspectief op onbewuste stress te kunnen presenteren. De meest veelbelovende aanpak hierbij is die van het gebruik van impliciete metingen in aanvulling op expliciete metingen.

About the author

Melanie van der Ploeg was born on June 3rd, 1986 in Leiderdorp, The Netherlands, and lived in Lisse throughout her childhood where she completed secondary school at the Fioretti College in 2004. After a leap year, she started with her Bachelor in Psychology at Leiden University. During her studies she actively participated in the university's community, which included a year as the board member of Educational Affairs of the student union Labyrint. She graduated in 2009. In line with her interest in the relationship between somatic diseases and psychological health, she started with the Master Medical psychology at Tilburg University in 2009. This two-year program offered her the opportunity to simultaneously work as an intern in a clinical as well as in a research setting. During her work at the department of Medical psychology of the ETZ Elisabeth in Tilburg, she provided individual and group therapy to patients with various medical conditions that faced psychological difficulties. She focused mainly on patients with cardiovascular diseases and contributed to the cardiac rehabiliation program (INFO and PEP module). For her research, Melanie participated in the project "Psychological Burden in Peripheral Arterial Disease". She pro-actively collected and processed data in collaboration with the department of Surgery at the ETZ Elisabeth in Tilburg. In addition to her studies, Melanie volunteered at the children's hospice Mappa Mondo in Waalre and the Alrijne Hospital in Leiden. Melanie graduated in 2011.

Based on her experiences from the clinical work, she aspired to further study the relationship between psychological stress and health. In 2012 she started her graduate work on the project 'Stress-related prolonged cardiovascular activity: The impact and changeability of stressful cognition without awareness'. In a series of experiments, as described in this dissertation, Melanie explored the role of psychological stress beyond self-report in health-relevant cardiovascular changes. In 2014 she visited The Ohio State University, Columbus (Ohio), USA, as visiting research scholar at the Emotions and Quantitative Psychology Lab and, amongst other activities, executed one of the experiments of her project (Chapter 3). Throughout her graduate work, Melanie was a teacher of several workgroups at Bachelor 2 and 3 level and supervisor of 15 theses at Master level. When she was finalizing her dissertation, she moved from Leiden to Prinsenbeek with her partner Ernest Pompe and their son Ludo Pompe (born in 2016). Melanie now works as a data-analyst at the municipality Oosterhout.

Publications

Articles

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Book chapter

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Data

Data published in this dissertation are available at https://osf.io/d6czx/.

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