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by

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ASSESSMENT OF PSYCHOLOGICAL AND PSYCHOPHYSIOLOGICAL CHARACTERISTICS INVOLVED IN THE MODULATION OF ENDOGENOUS PAIN CONTROL PATHWAYS AND IN THE INDUCTION OF PARADOXICAL PAIN

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"All that we are is the result of what we have thought.
The mind is everything.
What we think we become."

Hindu Prince Gautama Siddharta,
the founder of Buddhism,
563-483 B.C.

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Contents

1. Summary

The present doctoral thesis involves three experimental studies on pain processing and its modulation by psychological mechanisms. The first investigation focused on the relationship between associative learning aspects and the endogenous pain control system referred to as diffuse noxious inhibitory controls (DNIC) or conditioned pain modulation (CPM). The aim of the study consisted in uncovering whether descending pain inhibition may depend on specific environmental or circumstantial cues that have been linked to a reduction of pain sensations through associative learning in pain treatment contexts. A heterotopic noxious counter-stimulation (HNCS) was used to trigger the endogenous pain control system in the experimental context. For the study of the potential impact of associative learning on the pain-inhibits-pain phenomenon, a respondent (Pavlovian) conditioning procedure was realized during the HNCS stimulation. It could be shown that the repeated pairing of a phone signal (conditioned stimulus, CS) with the unconditioned tonic pain stimulus (UCS, HNCS) enabled the CS in the post-conditioning phase to generate a DNIC-like effect similar to the one induced by the tonic pain stimulus. The results demonstrated that learning processes are able to influence endogenous pain modulation processes and decreases in pain perceptions and reflex activity.

A thermal grill paradigm was used in the second and third study to examine the influence of psychological characteristics on the individual disposition to display the thermal grill illusion (TGI) of pain. First, the impact of pain-related personality traits like trait anxiety, pain catastrophizing, rumination, pessimism, expectancy of pain, suggestibility and interoceptive accuracy on inter-individual differences in paradoxical pain sensitivity was assessed. Second, the potential influence of dispositional self-regulation ability on illusive pain perceptions was measured. Vagally mediated heart rate variability (HRV) at rest was used as an index of individual self-regulatory strength. The results allowed identifying several psychological factors that are substantially affecting thermal grill-related perceptions. Mainly ruminative and interoceptive accuracy features increased the probability of paradoxical pain perceptions. The likelihood of the TGI elicitation was in addition significantly affected by the magnitude of the HRV-indicator respiratory sinus arrhythmia (RSA). Since thermal grill and neuropathic pain-related pain processing share common neural pathways, the identified psychological effects may be relevant in the context of pathological pain conditions.

2. Introduction

2.1 Theoretical background

Pain is a conscious experience resulting from nociceptive processing and signalling an objective presence or threat of tissue damage. Since the pain signal drives the organism to avoid injury, it is considered as essential for survival. Affective and motivational qualities are known to complement the sensory-discriminative determinant of pain (Ossipov, 2012; Rainville, 2002). The painful perceptions underlie subjective interpretations of the nociceptive input that are influenced by emotional, cognitive, pathological, genetic, or memory factors (Tracey and Mantyh, 2007). The Task Force on Taxonomy of the International Society for the Study of Pain (IASP; Merksey and Bogduk, 1994) has defined the highly subjective experience of pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". These aversive sensory and emotional properties of pain accentuate its warning signal character, which drives for a protective behavioural response required for potential injury avoidance or tissue damage reduction, as may be necessary to possibly guarantee survival (Tracey and Mantyh, 2007). Self-regulating processes controlled by the prefrontal cortex support a flexible somatosensory and affective adaptation to pain thus warranting homeostatic body states. Pain may however persist for longer periods (i.e. become chronic when it lasts more than three months) and its initial predictive and adaptive character looses its functional feature.

Surveys on chronic pain in Europe have revealed that 20% of the populations suffer from pain (Breivik et al., 2006). Statistics published by IASP and the European Federation of the IASP Chapters (EFIC) have indicated that about half to two-third of chronic pain sufferers are less or not able to pursue normal daily activities, to attend social contacts, to enjoy normal sleep, or to exercise. High emotional distress and worry are burdening consequences of long-lasting impairing pain and broadly affect human wellbeing. This large public health problem costs nations each year billions of dollar in medical treatment and lost productivity. Especially in developing countries where pain relief measures are only poorly available, the consequences on the patients' quality of life and the health care system are enormous. Meanwhile, IASP, together with the World Health Organization (WHO, Geneva, 2004) claimed: "pain relief should be a human right, a right to the highest attainable level of physical and mental health, whether people are suffering from cancer, HIV/AIDS or any other painful condition".

Since pain research has widened its scope over the last decades and pain has been conceptualized as a complex and multidimensional experience, chronic pain has been investigated from a biopsychosocial perspective, analysing the intricate relationships between bodily (e.g. genetic predispositions, neuroendocrine and -immune regulations), social (e.g. socioeconomic status, cultural and contextual differences) and psychological (e.g. negative emotions, learning processes, cognitive appraisal and coping styles) factors that underlie pain pathology and its modulation (Anton, 2009). The study of the underlying mechanisms of pain revealed that the relationship between the noxious input and output is not always linear. Environmental and psychological influences have recurrently been held responsible for the observed differences and the maladaptive interpretation of pain signals (Tracey and Mantyh, 2007). Despite the growing knowledge of pain and its treatment, many mysteries of pain chronicity and other pathological pain states still need to be solved.

The demonstration of the powerful influence of psychological features on both amplification and attenuation of pain perceptions captivated the author of the present thesis. For that reason the main interest of the current research was devoted to the assessment of the impact of psychological and psychophysiological aspects potentially affecting pain perceptions. Beneficial effects of learning processes and memory were analysed in relationship with the endogenous pain control mechanism termed as diffuse noxious inhibitory controls (DNIC). Several cognitive and emotional factors known to alter pain perception in clinical and experimental pain models based on supra-threshold noxious stimulation were investigated in association with the non-noxiously and only individually elicited thermal grill illusion of pain. Before explaining the psychological and psychophysiological variables and the experimental paradigms studied in the present research context, the neurophysiological and neuroanatomical underpinnings of pain processing will be described in the following chapter.

2.2 Central nervous system pathways involved in pain and pain modulation

2.2.1 From a 'hard-wired' to a plastic pain system

Neurobiological studies of nociception in animal models have provided important information on molecular components and neural structures involved in pain-related neural pathways. This knowledge has been complemented by investigations in humans on pain-related changes in the central nervous system (CNS), e.g. with functional magnetic resonance imaging (fMRI) and with electroencephalography (EEG) methods. The technological developments have demonstrated that pain is not simply a 'hard-wired' alarm system directly conveying its signals from the periphery to the spinal cord and the brain as proposed by Descartes (1644). Pain integration and modulation processes in the CNS have been uncovered and a large distributed network activated during spinal and supraspinal nociceptive processing has been elaborated (Brooks and Tracey, 2005; Tracey and Mantyh, 2007). The interacting brain structures have been described as pain "neuromatrix" (Melzack, 1999) or more commonly as "pain matrix". Cortical, limbic, midbrain, and medullary structures have been shown to participate in the brain matrix (see Figure 1). Autonomic and emotional regulation sites are also involved in the pain modulation system.

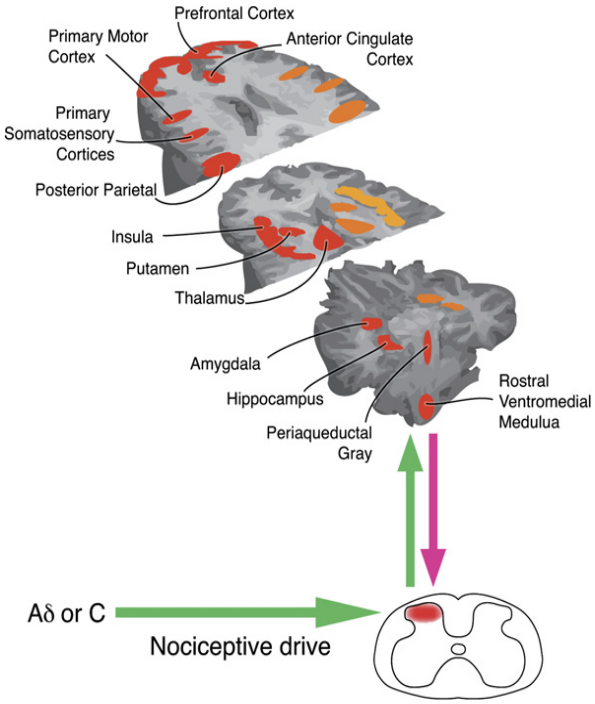

Figure 1: Neuroanatomy of pain processing. Main brain regions activated during a painful experience, highlighted as bilaterally active but with increased activation on the contralateral hemisphere (from Tracey and Mantyh, 2007).

2.2.2 Spinal segmental modulation of nociception

Noxious thermal, mechanical, or chemical stimuli applied at the periphery are detected by specific sensory neurons, the so-termed nociceptors. The nociceptive primary afferents project to the dorsal horn of the spinal cord and synapse with inter- or transmission neurons (Ossipov, 2012). Postsynaptic outputs of those neurons allow transmitting noxious signals from the spinal cord to the brainstem and higher order brain structures. Thinly myelinated Aδ-fibers and unmyelinated, slowly conducting C-fibers are the two kinds of nociceptors or primary afferent fibers that could be identified in nociceptive processing (Dubin and Patapoutian, 2010). With regard to the segmental spinal level, Melzack and Wall presented in 1965 the so-called gate control theory of pain, suggesting that in contrast to the historic picture of pain (Descartes, 1644), the pain system is subjected to significant modulation. The authors proposed that the non-nociceptive Aβ-fibers are able to interfere with the signalling of nociceptive fibers by activating inhibitory interneurons. In this way, a gate is closed, blocking the transmission of the noxious signal to the CNS and hence inhibiting pain. A particular merit of the gate control theory was that it integrated the potential implication of central control mechanisms modulating spinal nociceptive circuitry via descending pathways.

On the other hand, it could be shown later that a host of biochemical agents are able to sensitize nociceptors in the sense that neuronal activity gets enhanced and pain is strongly magnified (Costigan et al., 2009). These effects are referred to as peripheral sensitization and concomitant hyperalgesia at the subjective level.

In addition to the described nociceptors, post-synaptic nociceptive neurons may also become sensitized following enhanced or on-going peripheral input (for review see Woolf, 2007). This phenomenon has been labelled as central sensitization that may in turn also be involved in the maintenance and enhancement of pain.

A third mechanism involved in increases in pain sensitivity may be related to descending facilitatory inputs resulting from brainstem activity (Porecca et al., 2002).

2.2.3 Ascending pain pathways

There are two main ascending pathways that transmit nociceptive information to the brain (see Figure 2): the spinothalamic and the spinoreticular tract. The spinothalamic tract transmits contralateral information of pain, temperature, itch and crude touch to specific thalamic nuclei. Part of the ascending projections target brainstem structures like the periaqueductal grey matter (PAG) and the rostral ventromedial medulla (RVM). Signals are

then transferred to the somatosensory and cingulate cortex. The spinoreticular pathway is involved in the processing of emotional aspects of pain that promote action. The spinal projections of the spinoreticular tract mainly target the reticular formation of the brainstem. Information is then primarily sent to medial thalamic nuclei before being transferred to different sub-cortical and cortical areas.

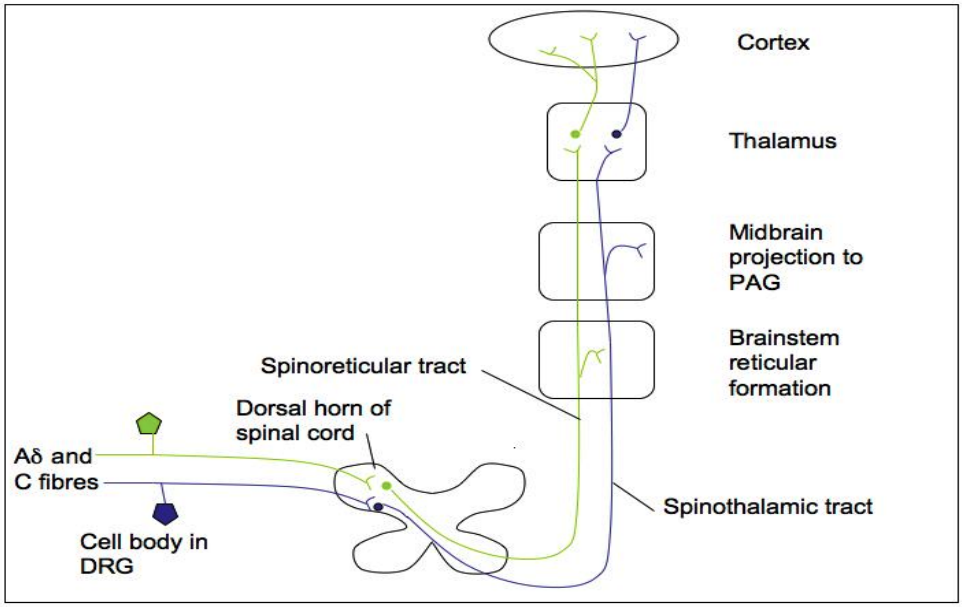

Figure 2. Ascending pathways. DRG = dorsal root ganglion, PAG = periaqueductal grey matter.

The spinal projections to the brainstem play an important role in the integration of homeostatic or autonomic conditions in pain processing (Tracey and Mantyh, 2007). It has furthermore been suggested that the brainstem is directly involved in the mediation of changes in pain perceptions and in the information transfer to the frontal cortex. The latter cortical area has recurrently been associated to emotional or cognitive self-regulation mechanisms engaged in homeostasis (Thayer and Lane, 2000).

2.2.4 Descending pain pathways

Over the last decades, it could be established that descending pain pathways allow regulating pain processing by inhibiting or facilitating spinal nociceptive transmission (Basbaum and Fields, 1984; Ren and Dubner, 2002). The RVM, the PAG, the hypothalamus, the amygdala, the insula, the anterior cingulate cortex (ACC), and the frontal lobe have been identified as main brain structures involved in the descending pain modulation system (Tracey and Mantyh, 2007; see Figure 3). The brainstem is considered as the main relay in the pain modulatory pathway that descends to the spinal cord. In

particular, the RVM and PAG are key features in both descending inhibition and descending facilitation of nociception, whereby the RVM is discussed as an important or even the final relay in the descending projection of facilitatory effects (Porreca et al., 2002). Descending pathways also control motor responses (reflexes) by transmitting signals to the ventral horn (Millan, 2002). Given that reflexes allow escaping harmful events thus bringing the exposure to a noxious stimulus to an end, the control of motor function is considered as an important part of nociception and its processing.

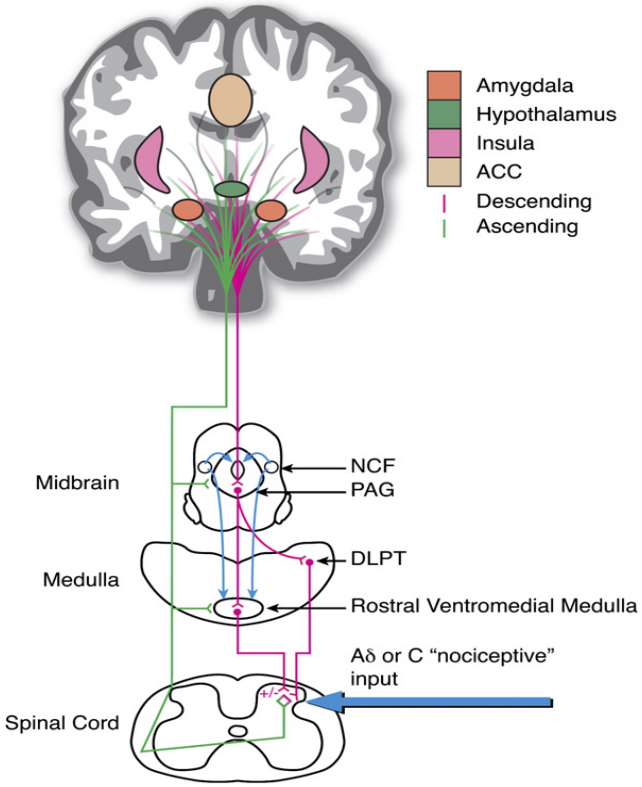

Figure 3. The Descending Pain Modulatory System. NCF = nucleus cuneiformis, PAG = periaqueductal gray; DLPT = dorsolateral pontine tegmentum, ACC = anterior cingulated cortex (from Tracey and Mantyh, 2007).

The axons of the descending supraspinal centres project to the spinal or medullary dorsal horn to modulate nociception in form of an increase or decrease of the magnitude of the pain perception (Fields and Basbaum, 2005). The PAG for instance has been identified as an important part of the top-down pain inhibitory circuitry that projects directly or indirectly to the spinal cord and is responsible for opioid- or environmentally mediated (i.e. fight-or-flight responses) analgesic effects (Fields and Basbaum, 2005). A link between the PAG, the amygdala, and cortical sites like the prefrontal cortex has been revealed in human imaging studies (Bingel and Tracey, 2008). Since the amygdala plays an important role in stress-related and emotional responses like anxiety, it has been suggested that this brain area acts in pain-related emotional and cognitive (e.g. executive functioning)

modulation to contribute to the efficient integration of pain. The prefrontal cortex supports the affective-cognitive regulation by means of inhibitory processes (Neugebauer et al., 2009). RVM-related pathways involving serotonergic and noradrenergic influences have been proposed as significant parts of the descending pain modulation system. The RVM collects neuronal signals from the PAG and is considered as a possible final relay in the descending antinociception circuitry (Fields et al., 1976). Projections from the RVM descend to the dorsal horn via the dorsolateral funiculus (DLF) to synaptically connect with primary afferent terminals, interneurons, and second-and third-order transmission neurons (Abols and Basbaum, 1981). The descending and ascending signal transmission of the RVM emphasizes its bidirectional role in pain modulation. Animal studies involving Pavlovian conditioning and expectancies have revealed that descending pathways are also engaged in the placebo and nocebo phenomenon (Benedetti et al., 2005; Ploghaus et al., 2003). Petrovic et al. (2002) stipulated that placebo analgesia is related to higher order cognitive networks and endogenous opioid systems. They observed that both placebo and opioid-related pain decreases went along with increased activity in the rostral ACC, respectively with an interaction between rACC and brainstem structures. It has furthermore been shown that descending pain control systems are recruited during pain relief generated with acupuncture (Liu et al., 2004).

So far, no anatomical separation of the neural structures supplying the descending inhibitory and facilitatory mechanisms has been observed (Millan, 2002), stressing the complexity of descending pain control. Anatomical and pharmacological studies suggested that while the two systems are distinct, they are simultaneously activated during acute pain states, thus warranting a kind of balance (Porreca et al., 2002). It has however been claimed that descending facilitation of spinal nociceptive input from the RVM is mainly responsible for the prolonged pain conditions in chronic pain states. The spino-bulbo-spinal loop also seems to support exacerbated pain behaviours produced by noxious (hyperalgesic) as well as also non-noxious (allodynic) peripheral stimuli in central neuropathic pain states. The physiological importance of pain facilitation is not understood in these pathological abnormal pain states where the initial injury does no longer exist and where persistent pain does not serve the purpose to protect the injured site by preventing its use.

Stress-induced analgesia

Stress-induced analgesia (SIA) is related to a form of descending control of spinal nociception leading to a decreased pain response. It constitutes an endogenous defensive mechanism protecting the organism from being impeded by an overwhelming pain experience in stressful or fearful life-threatening situations (Butler and Finn, 2009). In

terms of evolutionary mechanisms, SIA is considered as part of the fight-flight response. To study the effect of stress on pain inhibitory processes, pain models including unconditioned and conditioned SIA have been used (Butler and Finn, 2009). Although the exact mechanisms of action are not completely understood, endogenous opioids and cardiovascular reflexes (i.e. baroreflex sensitivity [BRS]) have been shown to be involved in SIA. Animal studies have indicated that supraspinal sites are essential in the spinopetal pain inhibitory circuitry (Watkins and Mayer, 1982). The results suggested that SIA is mediated by descending inhibitory pathways that refer to the amygdala and the PAG, exerting their effects via RVM projections to the spinal cord (Ossipov, 2010; see Figure 3). Basbaum and Fields (1984) have described the PAG as a major component of the stress-related pain suppression circuitry. In fMRI studies, activation of the primary and secondary somatosensory cortex, the anterior insula, and the rostral ACC was also identified during SIA (Yilmaz et al., 2010). Activity in the same brain networks could be observed during placebo- or DNIC-related hypoalgesia.

Diffuse noxious inhibitory controls [in humans: conditioned pain modulation]

DNIC constitutes an additional form of endogenous pain control system. The pain inhibitory mechanism can be regarded as a differential contrast-sharpening filter, in the sense that noxious stimuli on one body site may activate a kind of surround inhibition of ongoing painful stimulation at adjacent or distal body sites (Villanueva, 2009). The inhibitory effect of the pain-inhibits-pain phenomenon has been described as diffuse in nature (Le Bars et al., 1979). In humans, DNIC has also been referred to as counter-irritation analgesia or conditioned pain modulation (CPM; Yarnitsky et al., 2010). It relates to the fact that pain present in one region of the body may be attenuated by an additional pain stimulus applied to another body region. Classically, DNIC appears upon heterotopic noxious counter-stimulation (HNCS) and is increasingly used as a model to study human endogenous pain control mechanisms in both experimental (Reinert et al., 2000; Streff et al., 2011) and clinical studies (Van Wijk and Veldhuijzen, 2010). It has been suggested that the pain induced by new noxious stimuli is in this way better discriminated and is perceived as more important and more threatening for the organism as compared to previously existing pain sensations. Although being embedded in the neural pain control network and triggered by opioid mechanisms (e.g. periaqueductal grey), DNIC depend on basically stress-independent processing in the reticular formation of the brainstem. At spinal dorsal horn level, DNIC-related inhibition is limited to wide dynamic range (WDR) neurons (Le Bars et al., 1979). Ascending projections transmit nociceptive signals to structures like the amygdala, the thalamus, and the ACC. These higher order brain

structures then send information to brainstem nuclei like the PAG and RVM, which in turn provide the descending input to the spinal cord dorsal horn. This circuitry emphasizes the spinal-supraspinal-spinal feedback loop responsible for the DNIC-related pain reducing effects (Ossipov, 2010). Clinical data have accumulated, indicating that DNIC resp. CPM seem to be deficient in certain pain disorders (Lewis et al., 2012; Yarnitsky et al., 2008). Dysfunctional DNIC therefore might constitute a risk factor for the development of chronic pain.

Autonomic activity and descending pain regulation

Painful experiences are accompanied by higher sympathetic arousal, increases in blood pressure and baroreceptor stimulation. In healthy normotensive individuals, decreased pain sensitivity has been assessed in association with increased blood pressure. Reduced pain responses to acute pain have also been observed in clinical hypertension. Cardiovascular processes are considered as an important component of pain regulation. The functional interaction has been attributed to a homeostatic feedback loop that allows adaptively re-establishing arousal levels triggered by a noxious input (Bruehl and Chung, 2004). Ghione (1996) has proposed that the combination of hypertension and hypoalgesia depends on baroreceptor activity and endogenous opioids. A supraspinal loop involving vagal afferents (subserving the baroreflex) to the nucleus tractus solitarius (NTS) of the medulla oblongata is held responsible for the integration of autonomic actions in descending pain inhibition processing. The first synapse in the baroreceptor reflex system has furthermore been localized in the NTS (Randich and Maixner, 1984). It could also be shown that the central autonomous network (CAN) assists the reciprocal actions by coordinating cardiovascular and antinociceptive responses to environmental stimuli through specific brain regions (Ghione, 1996). Sympathetic and parasympathetic nuclei of the spinal cord seem moreover to be involved since they are strongly innervated by descending pathways. In this line, several findings have indicated that analgesic effects resulting from spinal cord processing may not be completely dissociated from cardiovascular functions given that changes in cardiovascular parameters are able to modulate descending influences on pain thresholds (Millan, 2002). Numerous animal studies have revealed an important role of endogenous opioids in the hypertension/hypoalgesia relationship (Bruehl and Chung, 2004). It was stipulated that endogenous opioids might be a prerequisite for the manifestation of the autonomic and antinociceptive interaction. However, given that findings of human studies have been more inconsistent in this regard, it may be suggested that the inverse association between blood pressure and pain sensitivity may not critically depend on the opioid system.

The adaptive relationship between cardiovascular and pain regulatory systems seems to be altered under chronic pain conditions. It has been suggested that changes in the activity of pain inhibitory and facilitatory pathways, endogenous pain control systems, or baroreflex sensitivity related to pathological pain states may contribute to an impaired blood pressure-related pain modulation system.

2.3 Psychological factors that influence nociceptive input and related pain perceptions

The identification of psychological features as major influences in subjective pain experiences provided further insight in the complexity and multidimensionality of pain processing and its underlying mechanisms (Wiech and Tracey, 2009). In neuroimaging studies, changes in neural activity during pain experiences disclosed how emotional and cognitive factors like anxiety, mood states, attention, expectancies, beliefs, and memories, are able to alter pain processing in humans (see Figure 4). It could be observed that the psychological aspects mainly acted in the framework of the descending pain modulatory circuitry and modified the strength and unpleasantness of pain perceptions either in terms of facilitation or inhibition of the noxious drive. Sustained activation of the descending circuits involved in facilitatory pain transmission has in particular been discussed in chronic pain states. Neuroimaging studies of the brainstem have shown that activity in the PAG changed according to the magnitude of e.g. distraction or attention to pain (Tracey et al., 2002). These changes in PAG activity correlated with changes in pain ratings. Valet and colleagues (2004) even provided evidence for top-down influences on the PAG. The researchers revealed connectivity between the cingulo-frontal cortex and the PAG in the framework of psychological gating of the respective pain modulation. In the elaboration of new treatments for chronic pain patients, psychological influences have been taken into account to a same extent than pathophysiological causes to ascertain the conciliation between peripheral and centrally mediated pain processing. Cognitive-affective and behavioural pain therapies are meanwhile used concomitantly with pharmacologically based methods (Crombez et al., 2005; Flor and Diers, 2007; Keefe et al., 1992).

2.3.1 Affect and pain

Anxiety and mood

The impressive effects of emotions and thoughts on current or expected pain perceptions may often be observed in daily life, whether in medical or ordinary contexts. An anxious or fearful, pain anticipating person risks feeling more pain during the treatment of an injury or another painful condition than somebody who is not particularly anxious. Inter-individual differences in anxiety and fear of pain could be associated to differences in pain sensitivity and neural pain processing (Ochsner et al., 2006). Although anxiety has been suggested generally to precede painful experiences, it has to be recognized that in

chronic pain states both aspects may be inversely correlated in the way that pain may lead to emotional disruptions, also called secondary pain affect (Price, 2000). The fMRI-based investigation of the effect of anxiety on pain processing has shown that the entorhinal cortex (i.e. responds in case of anxiety- and adversity-related behavioural conflict of the organism), the perigenual cingulate cortex (affective pain processing) and the mid insula (sensory pain processing) were highly activated during anxiety-related pain modulation (Gray and McNaughton, 2003).

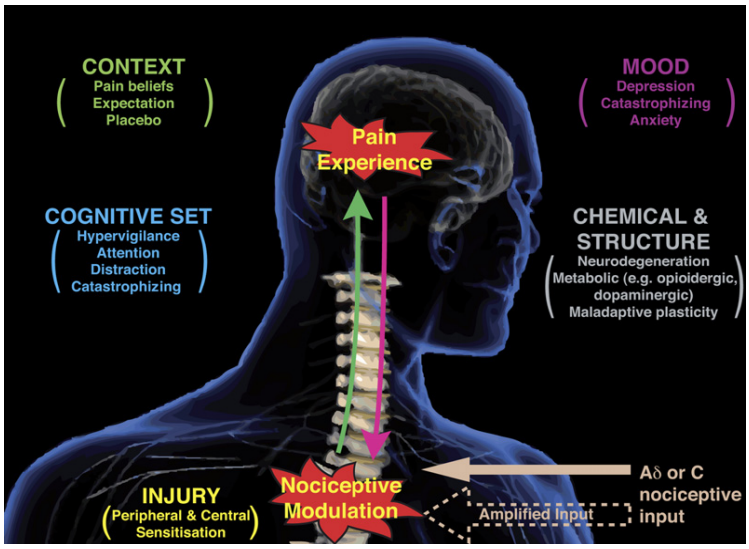

Figure 4. Main factors that influence nociceptive input and related pain perceptions (from Tracey and Mantyh, 2007).

It has been stipulated that emotional aspects rather influence the affective-motivational component of pain than the sensory-discriminative pain aspect. Pain intensity and pain unpleasantness are considered as separate components that may vary independently. Changes in pain intensity induced by hypnotic suggestions for instance generated other behavioural and neural responses than changes in pain unpleasantness (Wiech and Tracey, 2009). The differentiation between both pain aspects allowed elaborating cognitive-affective pain therapies and relaxation or hypnosis techniques that target changes in emotional influences. The beneficial impact of antidepressants primarily on pain unpleasantness has been assumed to be attributable to the anxiety reducing effect of these drugs (Dellemijn and Fields, 1994).

According to the motivational priming theory of Lang (1995), an emotional experience is shaped by two opposite motivational systems. On the one hand, appetitive stimuli activate the motivational system and cause positive emotions, whereas on the other hand adverse or potentially harmful stimuli initiate the defensive system, which motivates negative emotions. With regard to this model, experimental results have revealed that pain generally decreases when positive appetitive features like enjoyable music or pictures

influence the emotional state (Zelman et al., 1991). Interestingly, Rhudy and Meagher (2000) even observed an attenuation of pain when negative emotions were associated to noxious stimuli. It has been assumed that the decreased pain perceptions were a consequence of accompanying feelings of high threat and increased arousal that activated the stress-related pain inhibitory control mechanism SIA (Butler and Finn, 2009). Mood is considered as another important affective influence on pain processing and has been suggested as a mediator of the relationship between negative emotions and pain (Kenntner-Mabiala and Pauli, 2005; Zelman et al., 1991). Kenntner-Mabiala and Pauli (2005) showed that positive mood is able to reduce pain perceptions, whereas negative mood may increase painful sensations. Mood disorders like major depression or panic attacks are comorbid to chronic pain conditions. The relationship between depression and pain is underpinned by common biological pathways and transmitters and is considered as reciprocal (Bair et al., 2003). The exact neuroanatomical mechanisms underlying this association however still remain unspecified (Tracey and Mantyh, 2007). So far, it has been uncovered that the prevalence of pain in depressed patients and of depression in pain patients is higher then when both kinds of symptoms are evaluated separately. Depressed pain patients also express more pain complaints and impairments than non-depressed persons (Bair et al., 2003). Biased information processing, misinterpretation of bodily signals, and dysfunctional beliefs have been proposed as conveying negative mood in depressive individuals and emphasizing their pain (Beck, 2008). This close relationship between pain and emotional or cognitive influences underlines the strong interdependence of both kinds of psychological factors in pathological pain conditions (Wiech and Tracey, 2009).

The psychophysiological feature interoceptive accuracy

William James (1884) stated in his 'theory of emotion' that the perception of the physiological responses of our body to an emotional experience (e.g. changes in blood pressure or heart rate) shapes the following specific feeling. Antonio Damasio (1996, 1999) refined the theory of James with his 'somatic marker hypothesis'. The author proposed in his model that the perception of physiological reactions to a specific stimulus (i.e. changes in body and brain states, defined by the author as 'marker' signals) brings the importance of the respective stimulus to the front and orients the individual in his reasoning and decision-making. The marker signals are related to prefrontal cortices and bioregulatory processes. Although they are related to bodily states, the markers have their origin in the conscious or unconscious representation that the brain has of the body. Past negative experiences however may bias feelings and thoughts related to somatic perceptions and constitute a source of inappropriate or unadapted behaviour.

In recent years, the ability to accurately discern inner bodily states (e.g. the own heartbeats) has been recurrently investigated in the framework of interpersonal differences in the perception and sensitivity to internal visceral, vascular, or somatic changes. The psychophysiological feature has often been described as interoceptive accuracy (IA) or sensitivity (IS) and is considered as a stable trait (Tsakiris et al., 2011) or individual predisposition for more intense emotions (Wiens et al., 2000) like anxiety and catastrophizing (Critchley et al., 2004, Pollatos et al., 2007). Since the cognitive processing of bodily signals depends on an emotional appraisal of these signals, higher emotionality may interfere with the cognitive processing so as to render the cognitive appreciation of the interoceptive states dysfunctional and lead to a misjudgement of the bodily signals (Wölk et al., 2013) in terms of an over-report of somatic symptoms (Barsky and Borus, 1999). It could be shown in classical pain research that pain thresholds and tolerance levels were lower in those individuals who were more sensitive to internal body states (Pollatos et al., 2012). Pain sensations were also increased when IA was promoted by anxiety, catastrophizing, and high pain expectancy (Wiech and Tracey, 2009). It was observed that the anterior insula was activated during IS-related biased emotions, thoughts, and enhanced pain perceptions.

2.3.2 Cognitive characteristics affecting pain modulation and perception

Attention to pain, pain catastrophizing, rumination, and optimism

Closely related to emotional modulators of pain are cognitive factors that firmly shape pain processing and associated perceptions (Villemure and Bushnell, 2002; Wiech et al., 2008; Wiech and Tracey, 2009). The cognitive aspect attention for instance is able to alter both pain intensity and pain unpleasantness (Miron et al., 1989). It has often been reported that pain is perceived as more intense when an individual pays attention to the painful stimulus. Distraction, in contrast, allows drawing the attention to other sensory modalities, thus decreasing the painful sensations (Bushnell et al., 1999). Decreases in pain sensations were observed when attention to pain predominated as compared to pain avoidance (Keogh et al., 2000). These results suggest that the relationship between attention and pain is more complex than initially thought. The neural mechanisms underlying attentional pain modulation are not completely understood. Although neural activity has been observed in the afferent pain system during attention-related pain processing, it is assumed that the descending pain pathways are mainly involved in attention/distraction-related changes in pain sensitivity (Fields, 2000). High-resolution imaging of neurocognitive aspects of pain

perception showed that brainstem activity differs depending on an individual's level of attention or distraction. More distraction was paired with more activity in the PAG and was correlated with reduced pain ratings (Tracey et al., 2002). Further research supported these findings and confirmed moreover the existence of an anatomical association between the brainstem and cortical regions like the cingulo-frontal cortex (Valet et al., 2004) during attentional processes. Wiech et al. (2008) stated that attention mainly engages the dorso-lateral prefrontal cortex (DLPFC) and the ACC. In animal studies, these brain structures have interestingly been associated to descending pain modulation (Raz and Buhle, 2006).

In chronic pain patients, high (health) anxiety and fear of pain are often linked to cognitive features like pain catastrophizing or cognitive misinterpretations (Wiech and Tracey, 2009). When pain persists, ruminative thoughts about pain, pain magnification, feelings of helplessness, and pessimism are very likely to accompany catastrophizing (Edwards et al., 2006). It has furthermore been observed that there exists a relationship between catastrophizing and anticipation of pain, attention to pain, affective pain sensations, and motor control since brain activity was enhanced in the same areas during respective processing (Gracely et al., 2004). It was concluded that catastrophizing negatively affects pain perceptions by enhancing attention and anticipation of pain, as well as pain-related emotions. In pathological pain states, attention frequently turns into hyper-vigilance to pain and related contextual information and leads to maladaptive behavioural responses like hypochondrias. Cognitive-behavioural therapies allow successfully targeting this particular aspect (Keefe et al., 1992).

Expectancies and suggestibility

Attentional processes are currently conveyed by other cognitive mechanisms like expectations and beliefs about pain (Wiech et al., 2008). In clinical and experimental pain contexts, the extent of expected pain intensity plays an important role and modulates the magnitude of perceived pain (Hanssen et al., 2014; Price et al., 2008). Expectation has been shown to mediate analgesic placebo effects in the sense that pain ratings were reduced when participants consciously expected lower pain following prior conditioning (Benedetti et al., 2003; Colloca et al., 2008; Montgomery and Kirsch, 1997). Attentional (i.e. degree and frequency of somatic focusing), emotional (e.g. anxiety), and motivational (e.g. desires for symptom change) factors have been suggested as further important aspects in pain-related placebo responding. Positive placebo effects that are perceived as somatic improvements decrease the strength of the emotional and cognitive influences (Vase et al., 2005). Prior information or memory-related expectancies may modulate pain perceptions by biasing perceptual decision-making, the interpretation of the sensory information

(Wiech et al., 2014). Also closely linked to expectations about pain is the suggestibility characteristic (De Pascalis et al., 2002; Staats et al., 1998). It is widely accepted that pain may be lowered in highly suggestible participants following a suggestion of an efficient pain-relieving drug (De Pascalis et al., 2002). Opposite to the placebo effect is the nocebo phenomenon, described by Enck et al. (2008) as "the negative effect of placebo". When hostile expectancies or suggestions are delivered, the same psychological mechanisms as described above mediate active processes in the brain and initiate nocebo effects in terms of amplified pain experiences (Colloca and Benedetti, 2007; Enck et al., 2008; Tracey, 2010). Neurobiological investigations of the placebo effect have given insight into the complex interaction between powerful mental influences like expectations and underlying neural processes (Colloca et al., 2008; Zubieta et al., 2005). The authors have revealed that endogenous opioids mediate analgesic placebo effects related to expectancies. Placebo and opioid analgesia could furthermore be associated to higher activity in the rostral ACC, which in turn covaried with brainstem activity (Petrovic et al., 2002). With regard to adverse expectancies and nocebo effects, activation in several brain regions like the ACC, the PFC, and the insula could be uncovered with neuroimaging-based research (Keltner et al., 2006; Ploghaus et al., 1999).

Learning and memories

Learning and memory of prior experiences are important cognitive processes that shape the adaptive capacity and behaviour of healthy organisms. The competence in predicting the likelihood of a positive or negative event based on prior experiences qualifies individuals in adjusting their expectancies to external circumstances. Price et al. (1999) have demonstrated that remembered and expected pain levels are closely related and supposed to interact. Placebo-related pain ratings assessed concurrently and retrospectively to an experimental condition have shown that the retrospectively remembered pain intensities were rated much higher than the directly evaluated pain perceptions. Distorted memories of lower pre-treatment pain sensations diminished the magnitude of the subsequently expected and experienced pain. In contrast, memories of adverse pain experiences enhanced subsequent pain perceptions (Price et al., 2008). Placebo analgesia studies also referred to the role of associative learning or classical (Pavlovian) conditioning in some of the placebo responses. By repeatedly associating a neutral stimulus (conditioned stimulus, CS; e.g. a placebo pill) with an unconditioned stimulus (UCS; e.g. an active drug), the CS alone later triggers a similar physiological response than the UCS (Price et al., 2008).

A relationship between learning or memory processes and the development of chronic

pain has been established (Fordyce, 1976). Operant conditioning principles (i.e. reward and punishment) have been proposed as pain-behaviour-related influences. Factors like the escape from potentially painful threats, the avoidance of physical activity, drugs, attention in social contexts, have been considered as rewarding sources and pain behaviour maintaining aspects (Flor and Diers, 2007). These factors have been included in cognitive-behavioural pain therapies (Fordyce, 1973; Turk et al., 1983). Respondent learning mechanisms may also initiate or maintain unfavourable pain behaviours (Gentry and Bernal, 1977). Only by remembering a painful movement, fear of pain, muscle tension, and subsequent pain may be generated. Avoidance behaviour is motivated and the non-occurrence of pain powerfully reinforces the activity-avoiding attitude. Vlaeyen and Linton described this mechanism in their 'fear-avoidance model' (2000). In the Rescorla-Wagner model on Pavlovian conditioning (1972), the authors stipulated that an important mismatch between expected low and perceived high pain or vice-versa would determine the strength of the learned UCS-CS association respectively of the conditioned response. Surprising outcomes and memories related to these results would positively support learning processes as compared to predicted outcomes. Since chronic pain strongly bases on associative learning aspects, it has been suggested to integrate the Rescorla-Wagner approach in new treatments for chronic pain (Ploghaus et al., 2000).

In recent years, pain research has focused on the so-called pain memory and the underlying neural plasticity (e.g. peripheral and central sensitization) in the nociceptive system (Song and Carr, 1999). Central sensitization (e.g. hyperalgesia) and short or long-term potentiation processes have been considered responsible for creating a memory trace after the initial noxious stimulation had subsided and rendering the nervous system more excitable to subsequent stimulation (Arendt-Nielsen et al., 2008). Loeser and Melzack (1999) stipulated that learned experiences might play an adverse role in pain experiences when modifying interactions between the peripheral input and the neuromatrix. The brain and its pain memories would in consequence be able to generate pain even in the absence of a noxious input. Neuroimaging studies have revealed that the pain-related rACC also plays a role in pain-related learning and contextual pain memory (Ossipov, 2012).

2.3.3 Self-regulation

Over the last decade, self-regulation has become a prominent feature that has been thoroughly studied in the framework of human self-control and homeostasis. Emotional, cognitive, physiological, and behavioural self-regulation have been proposed as key mechanisms (Park and Thayer, 2014; Segerstrom and Solberg Nes, 2007; Solberg Nes et

al., 2009; Thayer et al., 2000, 2009, 2012) in the stable maintenance of a "harmonious balance of the elements" as Hippocrates (460 – 375 BC) denoted the state of good health. Walter Cannon stipulated in 1932 that several interacting and automatic bodily mechanisms like e.g. body temperature or autonomous neural processes facilitate the flexible adaptation of the body to challenging internal and external demands so as to guarantee a healthy state of physiological stability. The ability to self-regulate allows flexibly controlling for bodily states, functions, and behavioural processes while adapting to conscious or unconscious emotions, thoughts, or motivational influences (Park and Thayer, 2014). Especially external or internal emotional incentives seem to challenge the individual's self-regulatory capacities since they may affect executive functions like planning, decision-making or motivational aspects like goal-oriented behaviour (Solberg Nes et al., 2009).

Since pain has been discussed as a homeostatic emotion or behavioural drive like hunger or thirst (Craig, 2003), it is accepted that the regulating actions are also promoted during pain states thus warranting adaptive behaviour in the face of noxious challenges. To satisfy the organism's homeostatic drive for an equilibrated body condition, a flexible and effortful coping accompanies the behavioural disengagement from the negative sensory-affective pain state (Appelhans and Luecken, 2008; Craig, 2003). Deficits in self-regulation have been related to reduced executive functioning and chronic pain conditions (Solberg Nes et al., 2009). Investigations on state self-regulation ability in clinical and experimental pain models with suprathreshold noxious stimulation revealed that higher pain sensitivity is in general linked to lower self-regulation capacity (for review see Koenig et al., 2014). The affective-discriminative component of pain has also been considered in the context of homeostatic processes (Cacioppo et al., 1999). It was stipulated that pain unpleasantness is an emotional state and a homeostatic challenge that, by its strong association to the negative valence of the pain stimulus and the ensuing high arousal, motivates the subsequent behavioural detachment from the noxious input. Depressive symptoms have been shown to disrupt emotional self-regulation and to enhance pain unpleasantness (Berna et al., 2010).

The neural underpinnings of self-regulatory processes could be identified in the prefrontal cortex (Thayer et al., 2009, 2012; Wiech et al., 2006). The neural network in the medial PFC warrants the regulation of affective, cognitive, cardiac, physiological, and behavioural actions by means of inhibitory processes. Attentional or motivational processing for instance is controlled via the inhibition of inopportune information or emotions. A correlation has been found between prefrontal hypo-activity and self-regulation deficiencies. In daily life, self-regulatory top-down modulation supports flexible adaptation to contextual needs. In threatening or uncertain situations, hypoactive prefrontal

activity allows disinhibiting sympatho-excitatory circuits to efficiently mobilize energy requirements. Under conditions of anxiety, the prefrontal hypoactivity will prevent inhibitory neural processes thus enhancing deficits in working memory and executive function (e.g., Thayer and Friedman, 2004). On the other hand, frontal hypo-activity may be related to behavioural dysregulation and psychopathological disorders like attention deficit hyperactivity disorder, obsessive-compulsive disorder or mood disorder (Fassbender and Schweitzer, 2006). With regard to the adverse influence of negative beliefs about pain, self-control has become a noticeable characteristic in cognitive-behavioural pain therapies. It is considered that the positive change in a pain patient's beliefs on his self-efficacy or self-control and coping capacities when confronted with painful challenges will enable him to reappraise and better control his pain. fMRI-studies have shown that the analgesic effects of self-controlled thoughts and emotions are concordant with higher activity in anterior cingulate and prefrontal cortices (Wiech et al., 2008). As seen from the developmental perspective and in the context of the prefrontal structural properties, self-regulatory ability has been proposed as a rather stable trait over time. Modulations of self-regulation states are completed via motivational or situational influences.

In the context of cardiovascular regulation, cortical mechanisms also modulate the activity of the vagus nerve. It has been revealed that there is a relationship between the mPFC pathways and the central autonomous network (CAN), a neural system responsible for visceromotor, neuroendocrine, and behavioural homeostatic processes (Benarroch, 1993; Thayer and Lane, 2000). The CAN plays a key role in the reciprocal cortico-cardiac interactions required during flexible and homeostatic adaptation of the organism to situational demands. Subcortical structures like the amygdala, anterior cingulate cortex, insula, hypothalamus and brainstem nuclei have been associated with the CAN (Thayer et al., 2009). The PFC exerts a tonic inhibitory control on the amygdala and its influence in diverse physiological, autonomic, or endocrine regulation processes. Thayer and Lane (2000) included the CAN in the neurovisceral integration model (NVI) and proposed it as a functional unit regulating psychological and physiological control processes via the described neural circuitry and related inhibitory processes. The NVI refers to psychological and physiological regulation in the context of an adaptive homeostasis of bodily conditions to environmental demands (Thayer and Lane, 2000). The regulation activity is described as being supported by neural structures comprising the mPFC, several subcortical areas, and the CAN (Benarroch, 1993; Thayer and Lane, 2000; Thayer et al., 2009, 2012;). It has been stated that top-down inhibitory influences of the prefrontal areas modulate subcortical activity of the amygdala and related affective processes and are involved in the regulatory mechanisms.

3. Empirical studies

Introduction

Several of the previously described psychological variables have been analysed in the framework of the present PhD-thesis and are described in the following paragraphs with regard to the respective experimental pain paradigms. An HNCS-related pain model and a respondent conditioning procedure were used to investigate the potential pain alleviating influence of learning processes on the endogenous pain control system DNIC in our laboratory. A thermal grill paradigm served in the second and third study for the elicitation of the thermal grill illusion (TGI) and the identification of personality traits that explain the observed inter-individual differences in the perception of the TGI.

Since memory or learning effects have not only been related to increased but also to attenuated pain, the study of psychological influences like associative learning processes possibly modulating pain found great interest in the first study of my PhD-project. On the one hand, pain memories are considered as important pain maintaining factors in the development of chronic pain. They may be processed at the conscious or unconscious level and guide subsequent pain experiences and behaviours (Flor and Diers, 2007). The resulting increased pain sensitivity may even be enhanced by further learning effects. On the other hand, learning and memory in association with endogenous pain modulation processes have been postulated as possible explanations for the observed long-term hypoalgesic effects associated with non-pharmacological pain management techniques like transcutaneous electrical nerve stimulation (TENS), acupuncture or placebo treatments (of the order of days-weeks; Dhond et al., 2007; Widerström et al., 1992). When considering that the duration of SIA- or DNIC-related endogenous opioid effects fit the time course of drugs, it had to be admitted that endogenous pain modulation processes could not have influenced alone the persisting pain improvement. The pain-inhibitory effects observed in experimental investigations employing HNCS were also only of short duration (less than 15 min; Villanueva and Le Bars, 1995). Associative learning in terms of respondent or classical conditioning was suggested to allow bridging the observed time gap and account for the sustained DNIC-related hypoalgesia over time (Carlsson, 2002; Price et al., 1984). It seemed conceivable that situational cues like aspects involved in the therapeutic procedures (e.g. apparatus, white coat, therapeutic environment, etc.) could mutate from a neutral to a conditioned stimulus and by being remembered maintain the effects on the endogenous pain-inhibiting processes over a longer period. Analgesia-related operant

(Becker et al., 2008; Flor et al., 2002a) and respondent conditioning processes (Flor et al., 2002b) have already been investigated. Whilst Herta Flor and colleagues (2002b) analysed the role of associative learning mechanisms in stress-induced analgesic effects, the study of the same learning factors in DNIC-induced pain outcomes remains so far more or less elusive.

A preliminary study realized at the University of Luxembourg (unpublished) had revealed a potential conditionability of HNCS-induced hypoalgesia by pairing a heterotopic trigger stimulus with a neutral acoustic stimulus. It had been shown that repeated pairing of an unconditioned tonic pain stimulus (UCS; immersion of non-dominant hand in ice-cold water) with an initially neutral conditioning stimulus (CS; acoustic tone) was able to subsequently inhibit phasic stimulation-related (contact heat stimuli applied to the dominant forearm) pain perceptions, thus to produce a HNCS-induced hypoalgesia as an unconditioned response (UCR). Interestingly, the conditioning effect was not only confined to the sensory-discriminative component of pain, but manifested itself also in the autonomic pain component (i.e. less pronounced heart rate acceleration). The aforementioned study used verbal pain behaviour (i.e. subjective pain ratings) and cardiovascular responses as dependent variables. In the current study, we investigated whether the observed conditioning of HNCS-induced pain inhibition can be replicated and does also affect nocifensive reflexes like the RIII flexion (or withdrawal) reflex activity of the biceps femoris muscle and the frowning reflex activity of the corrugator supercilious muscle (Craig, 1985). Both reflex measures were included in the study to include more objective pain measures.

The expression ‘conditioned pain modulation’ has not been used here as a synonym of HNCS or DNIC since the use of one and the same word (i.e. conditioned resp. conditioning) in the simultaneous description of pain inhibition and learning methods might lead to confusion.

An interesting and at the neurophysiological level thoroughly studied phenomenon, is the so-called thermal grill illusion of pain (TGI), also termed paradoxical or synthetic pain. The TGI relates to the phenomenon that synchronously touching innocuous cold and warm (e.g. juxtaposed cooled or heated metal rods in a Peltier-driven thermal grill device or glass tubes in a water-bath driven thermal grill) produces an intense painful sensation that is most often qualified as heat or burning pain. The peculiarity of these thermal stimuli is that when they are touched independently, no pain is experienced.

Paradoxical pain has been postulated to rely on a central integration of the thermal input and interaction of the thermo-sensory and nociceptive system (Bouhassira et al., 2005; Leung et al., 2005). In their ‘central disinhibition theory’, Craig and Bushnell (1994) stated

that the painful grill illusion may be explained by the disinhibition of the cold-evoked activity of polymodal nociceptive lamina I spinothalamic neurons (activation by polymodal C-nociceptors) ensuing a reduced normal cold-evoked activity of thermoreceptive lamina I spinothalamic neurons (activation by Aδ cooling thermoreceptors) during simultaneous application of warm stimuli in the thermoreceptive, but not in the nociceptive neurons.

The thermal grill paradigm has regained new research interest during the last years since it has been demonstrated that common neural mechanisms are shared during the processing of the TGI and central neuropathic pain (Craig, 2008). The use of the thermal grill as an experimental tool allowed identifying the neurophysiological and neuroanatomical mechanisms underlying the TGI resp. central pain. In this way, new insight could be gained on dysfunctional interactions between feelings of temperature and pain (Craig, 2008; Kern et al., 2008). Other studies focused on neuropathic pain sensitivity when influenced by mood disorders (Boettger et al., 2011; Piñerua-Shuhaibar et al., 2011). Thermal grill-related stimulation parameters like stimulation temperature combinations, duration, or distances between the cold and warm bars, were moreover analysed to gain information about the frequency, the quality, and the intensity of the elicited TGI (Bouhassira et al., 2005; Boettger et al., 2013). The findings of these studies interestingly revealed inter-individual differences in paradoxical pain sensitivity. It could be observed that the painful TGI is only perceived by about one third of a sample (Bouhassira et al., 2005). Given that the reasons for the observed individual differences in the perception of the TGI have not been systematically investigated, this issue became one of the focal points of the present thesis. It was hypothesized that pain-related personality traits, identified as pain enhancing features in experimental or clinical pain models *basing on noxious input*, might possibly also play a role in *non-noxiously* elicited paradoxical pain.

In the third study, the thermal grill paradigm was used to measure the extent of self-regulation capacity in responders and non-responders to the thermal grill stimulation. Since deficits in emotional and cognitive self-regulation ability and related prefrontal activity have been associated with affective instability, impaired coping and reappraisal processes, self-regulatory fatigue, stress, increased pain sensitivity, chronic pain conditions and other health problems, it seemed conceivable that differences in self-regulation capacity might be responsible for the observed differences in paradoxical pain sensitivity. Vagally mediated heart rate variability (HRV) at rest was assessed as an indicator of tonic vagal activation respectively trait self-regulation capacity (Koenig et al., 2014).

3.1 Study 1: Beep tones attenuate pain following Pavlovian conditioning of an endogenous pain control mechanism

Abstract

Heterotopic noxious counter-stimulation (HNCS) is commonly used to study endogenous pain control systems. The resulting pain inhibition is primarily based on spinal cord-brainstem loops. Recently, functional imaging studies have shown that limbic structures like the anterior cingulate cortex and amygdala are also implicated. Since these structures are involved in learning processes, it is possible that the HNCS-induced pain inhibition may depend on specific cues from the environment that have been associated with pain reduction through associative learning. We investigated the influence of Pavlovian conditioning on HNCS-induced pain inhibition in 32 healthy subjects by using a differential conditioning paradigm in which two different acoustic stimuli were either repeatedly paired or unpaired with HNCS. Series of noxious electrical pulse trains delivered to the non-dominant foot served as test stimuli. Diffuse noxious inhibitory control (DNIC)-like effects were induced by concurrent application of tonic HNCS (immersion of the contralateral hand in ice water). Subjective pain intensity and pain unpleasantness ratings and electromyographic recordings of the facial corrugator muscle and the nocifensive RIII flexion reflex were used to measure changes in pain sensitivity. HNCS induced significant pain and reflex inhibitions. In the post-conditioning phase, only the paired auditory cue was able to significantly reduce pain perceptions and corrugator muscle activity. No conditioned effect could be observed in RIII reflex responses. Our results indicate that the functional state of endogenous pain control systems may depend on associative learning processes that, like in the present study, may lead to an attenuation of pain perception. Similar albeit opposite conditioning of pain control mechanisms may significantly be involved in the exacerbation and chronification of pain states.

Beep tones attenuate pain following Pavlovian conditioning of an endogenous pain control mechanism

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Introduction

Endogenous pain control systems include mechanisms like descending inhibition, stress-induced analgesia [1] and diffuse noxious inhibitory controls (DNIC) [2]. In humans, DNIC has also been referred to as counter-irritation analgesia or conditioned pain modulation [3]. It relates to the fact that pain present in one region of the body may be attenuated by an additional pain stimulus applied to another body region. Classically, DNIC appears upon heterotopic noxious counter-stimulation (HNCS) and is increasingly used as a model to study human endogenous pain control mechanisms in both experimental [4,5,6] and clinical studies [7,8].

DNIC-related analgesia was originally studied in animals by focusing mainly on spino-bulbo-spinal pathways [9,10]. More recently, functional magnetic resonance imaging (fMRI) studies in humans have shown that cerebral structures like the anterior cingulate cortex and the amygdala contribute to HNCS-induced hypoalgesia [11,12]. Interestingly, these limbic regions have also been found to be involved in learning processes [13,14]. It is thus conceivable that endogenous pain control systems may be influenced by cues from the environment that have been acquired through associative conditioning. The finding that stress-induced analgesia can be successfully conditioned [15] provides further support for the assumption that associative learning processes may influence pain processing mechanisms and hence possibly play a role in the development of chronic pain (for review see [16]).

Of particular interest within the phenomenon of HNCS-induced hypoalgesia is the enduring effect of therapeutic procedures like acupuncture or transcutaneous electrical nerve stimulation (TENS). DNIC-like processes are thought to mediate at least partially this effect [17,18]. However, DNIC-related pain inhibition only lasts several minutes [10] whereas the therapeutic efficacy of acupuncture and TENS may persist for hours or even days [19,20]. Hypothetically, this discrepancy may be attributable to associative learning of initially neutral cues from the environment that may serve as conditioned stimuli for the induction of long-lasting hypoalgesic effects.

The present study was aimed to demonstrate that HNCS-induced pain inhibition can be successfully conditioned. To measure endogenous pain inhibition based on the counter-stimulation and the conditioning procedure, we collected subjective pain intensity and pain unpleasantness ratings and objective physiological parameters of nociception and hyperalgesia like electromyographic (EMG) activity related to facial corrugator muscle- (frowning or brow lowering reflex) and to nocifensive RIII flexion reflex (withdrawal reflex) activity of the biceps femoris muscle, respectively. The corrugator muscle activity is mostly recorded as a measure of primarily negative facial expression while experiencing

pain [21]. The RIII reflex is correlated with pain threshold and is commonly used as a tool for the study of pain mechanisms and for the evaluation of treatment [22,23,24]. Since we could confirm that psychophysical and psychophysiological pain-related responses were attenuated following the respondent conditioning procedure, the above mentioned main goal of the study was achieved.

Material and Methods

Participants

Participants were recruited among the students and the staff of the University of Luxembourg and received financial compensation. Volunteers with a history of chronic pain, cardiovascular, dermatological, neurological, and psychiatric disorders were excluded from the study. Only those subjects tolerating the cold pressor test for at least 1 min during the assessment of their pain threshold and tolerance level to ice-water immersion prior to the experiment (cf. experimental protocol) were allowed to participate in the study. At the same time point, the participants had to reach pain intensity ratings of at least 2 on a verbally anchored numerical rating scale (NRS; 0–10; 0 = *no pain*, 10 = *worst pain imaginable*; pain ratings were done by increments of 1.0 or 0.5 decimals on the 0–10 NRS) to make sure that the cold pressor test could be used as HNCS. They also had to show an HNCS-induced pain reduction of at least 5% in the pre-conditioning baseline (BL) 2 (i.e. the BL2 stimulation block was characterized by three electrical stimulation series and a simultaneous application of the cold pressor test serving as HNCS) and had to tolerate electrical stimulation during the RIII threshold delineation. Since hypertension has been shown to be associated with lower pain sensitivity [26], only normotensive participants were included (< 140 mmHg systolic and 90 mmHg diastolic; manometrically assessed).

Among the 53 recruited participants, 21 subjects could not participate in the experiment, either because the DNIC-effect could not be triggered during BL2 or because they did not tolerate the electrical stimulation intensity during the RIII threshold assessment. A final sample of 32 healthy drug-free subjects (11 female and 21 male; 29 right- and 3 left-handed; age range 18–39 years, median = 23 years) gave informed written consent to participate in the study. As a cover story, participants were informed that they were taking part in an experimental study investigating the relationship between pain and cardiovascular parameters and that the auditory cues merely indicated the duration of the stimulation sequences. Experimental protocols are in line with ethical guidelines of the International Association for the Study of Pain (IASP) [25] and were approved by the National Research Ethics Committee (ref. 1102-59).

Material and equipment

Phasic electrical stimuli were provided by a pulse generator (A 310 Accupulser, World Precision Instruments, USA) and were delivered through a constant-voltage-stimulator (Unipolar Pulse STM200, BIOPAC Systems, Inc., USA) [27,28]. Stimulation was applied through two convex tin electrodes (diameter 0.5 cm; EL350S; BIOPAC Systems Inc., USA) placed 2 cm apart on an acrylic bar. The electrodes were fixed with an adhesive strip posterior to the ankle of the contra-lateral (non-dominant) foot, at the height of the sural nerve. The ankle was flexed at 90° and the knee at 130°. Skin impedance at the foot was measured with a Multimeter Analog HM-120 BZ (Hung Chang Co. Ltd.; Seoul, South Corea) and had to remain below 10 kΩ.

The RIII reflex threshold was assessed with a modified staircase method [23,29]. Single electrical pulses (1 ms) of increasing strength (ranging from 0.5–3 V) were delivered until the first RIII reflex response emerged. The threshold intensity was considered to be reliable when 2–3 repetitive stimuli yielded stable EMG responses exceeding an integrated area of 100 μV*s [30]. RIII reflex-eliciting stimulation intensity was individually adjusted and fixed at max.110% reflex threshold to preclude pain at tolerance level during the wind-up procedure.

During the experimental trials, electrical stimulation consisted in rectangular pulse trains (pulse width: 25 ms, repetition rate: 200 Hz, 5 pulses of 1 ms each) [31,24]. These pulse trains were presented in series of four at an inter-stimulus interval (ISI) of 500 ms to induce temporal summation of the nocifensive RIII reflex [31,23,24] and psychophysical pain responses. This paradigm was chosen to have a pain marker that is not influenced by distraction effects [32]. The duration of one wind-up series was 1.6 s. In each stimulation block, three of these series were delivered at intervals of 25 s to avoid habituation of the stimuli. The total duration of the three stimulation series and the respective intervals was ± 55 s. A detailed overview of the electrical stimulation paradigm is displayed in Fig. 1B. Specimen of RIII-signal recordings are depicted in Fig. 1A.

HNCS consisted in the immersion of the dominant hand up to the wrist in ice water for 75 s [33]. The water was kept at a constant temperature of approx. 2° C using an external chiller (Aqua Medic GmbH, Germany). For the tepid water control condition, a commercially available submergible heater and an external digital control device (T-controller T2001 HC, Aqua Medic GmbH, Germany) were used to keep water temperature constant (32 ± 2 °C).

All electrical and acoustic stimuli were controlled via E-Prime presentation software (Psychology Software Tools Inc., USA).

Psychophysiological recording

Physiological activity was continuously recorded with an MP150 Data Acquisition System (BIOPAC Systems Inc., USA).

EMG activity of the facial corrugator supercilious muscle (for measuring frowning responses) and the biceps femoris muscle (for assessing RIII reflex responses) was recorded with an EMG100C amplifier (both with 500 Hz low and 10 Hz high-pass filter and a signal gain of 500). For the RIII reflex measurement, two shielded disposable and pre-gelled Ag-AgCl electrodes (diameter 24 mm, H124SG, Kendall Electrodes) were placed at the non-dominant upper leg, over the short head of the biceps femoris muscle (distance between electrodes 20 mm) [30,34]. Recordings were only initiated when the impedance was below 5 kΩ. The same type of electrodes was also used for corrugator muscle activity recording. The electrodes were fixed 15 mm apart over the left eyebrow in parallel to the muscle midline [35]. Before application of the EMG recording and stimulation electrodes, the skin at the leg, foot, and forehead was cleaned with ethanol and abraded. The electrode placement area on the leg was shaved when necessary.

Beat-to-beat BP was measured by analyzing the timing and amplitude of the primary left ventricular ejection pulse as well as the arterial pulse reflections at the wrist of the non-dominant arm (NIB P100A; Medwave Vasotrac APM205A). A standard precordial lead II electrocardiogram (ECG) (ECG100C; 0.5 Hz high pass filtering, R-wave output mode, signal gain 500) was performed using disposable pre-gelled Ag-AgCl electrodes (diameter 35 mm, EL502, Biopac Systems) placed below the right clavicle and below the left lower rib. Pulse and ECG recordings were used to compute continuous HR.

Electrodermal activity was assessed with two domed Ag-AgCl electrodes (diameter 6 mm, SS3LA, Biopac Systems) filled with isotonic paste (containing 0.5% saline in a neutral base). The electrodes were attached to the mid-phalanx of the third and the fourth finger of the non-dominant hand. The signal was processed through a constant voltage (0.5 V) coupler (GSR100C, 1.0 Hz low pass filtering, signal gain 5 μS/V).

Subjects were grounded through an unshielded disposable Ag-AgCl electrode (diameter 24 mm, H124SG, Kendall Electrodes) positioned at the midpoint of the left calf (non-dominant leg.

Experimental protocol

The protocol corresponded to a randomized controlled trial. Experimental sessions were based on a differential conditioning paradigm and comprised a pre-conditioning (i.e. baseline), a conditioning (i.e. acquisition) and a post-conditioning (i.e. test) phase. The experimental stimulation blocks were identical for all experimental groups, except for the differential procedure during the conditioning phase. The experimental procedure is

summarized in Fig. 1A. Each subject participated in a single session lasting about two hours. Experiments took place in a temperature-controlled room (approximately 22° C) and were all performed by the same investigator.

Prior to the beginning of the experiment, the participants' pain threshold and tolerance level to ice-water immersion was measured. The subjects immersed their dominant hand into the ice water bath over a period of 1 minute and rated the induced pain intensity on a 10-point NRS in 10 s intervals. Subsequently, all electrodes and sensors were attached (see material and equipment section). Participants were then given a 5 min rest before the RIII reflex threshold was determined. For this purpose, single electrical pulses of increasing intensity were applied until the electrical stimulation reliably induced an RIII reflex. Pain intensity ratings of the applied pulses were assessed simultaneously.

Whereas the objective psychophysiological responses to the phasic electrical test stimuli were continuously measured online during the pre- and post-conditioning phases, the subjective pain intensity and pain unpleasantness perceptions were assessed only at the end of each electrical stimulation series (wind-up; 3 x per stimulation block), but throughout the whole experiment.

In the pre-conditioning phase, all participants were submitted to two baseline measurements (BL1 and BL2, see Fig. 1A). BL1 involved three electrical stimulation series and BL2 was characterized by a simultaneous application of the cold pressor test serving as HNCS.

For the conditioning and post-conditioning phases, subjects were randomly assigned to the test group (N_1 = 16) or to the control group (N_2 = 16). Subjects in the test group were exposed to a differential conditioning procedure. Here, two sounds of different frequency were used as conditional stimuli (CS). A common dial phone signal, consisting of a 344-Hz continuous wave, was considered as sound A, whereas a busy phone signal, made up of a 600-Hz interrupted wave, was applied as sound B. Each sound was presented with 65 dB via headphones. To test for response generalization, CS+ salience and habituation, these initially neutral acoustic stimuli were presented in counterbalanced order with regard to their use as CS. Half of the participants in the test group (N_{1A} = 8) did consequently receive sound A as CS– and sound B as CS+, whereas the attribution of the tones was reversed in the other half of the test group (N_{1B} = 8), sound B serving as CS– and sound A as CS+. Participants in the test group were randomly assigned to one of these two conditions. During the conditioning phase, the acoustic stimuli were either paired (CS+) or unpaired (CS–) with repeated immersion of the dominant hand into the ice or tepid water bath. In the test group, innocuous tepid water immersion was consistently unpaired with CS– and

noxious ice water immersion was always paired with CS+ (see experimental protocol in Fig. 1A).

The conditioning phase started 15 min after the baseline assessments BL1 and BL2 to allow HNCS-induced inhibitory effects to fade out [24]. Subjects in all experimental groups had six neutral (tepid water immersion) and six HNCS (cold water immersion) blocks. Tepid water was used as control condition and was always applied before cold water in order to avoid a potential activation of counter-irritation mechanisms [24]. Tonic noxious stimulation (ice water, HNCS) not only served as trigger to induce DNIC-like effects, but also as unconditioned stimulus (US). Phasic noxious electrical pulses that were applied to the contralateral foot were used as test stimuli. According to the respondent conditioning model, pain sensation- and reflex response alterations upon HNCS constituted the unconditioned response (UR). The inhibition of nociceptive processing induced by CS+ during the post-conditioning phase was considered as the conditioned response (CR).

A bubble sound (50 dB) signaled when to immerse the dominant hand into the water bath. The ice or tepid water exposure as well as the auditory stimulations (CS) always persisted for 75 s. These thermal and acoustic stimuli were initiated and terminated simultaneously. Since pain sensations during the cold-water immersions do not occur immediately but typically show a delay [36], electrical stimulation series (3 wind-up) were applied 20 s after the start of the tonic pain stimulation and lasted in total 55 s. Participants were instructed to lift their hand out of the water bath during inter-trial intervals (period of 45 s). Together with the thermal/acoustic stimulation duration (75 s) and the related ISI (45 s), 120 s (2 min) were required for one stimulation block.

Contrary to the test group, participants in the control group (N_2 = 16) were not subjected to any associative learning procedure, but only to unpaired pain stimulations. In order to account for potential confounding (e.g. distraction and alertness due to the presentation of the auditory cues) and sequence effects (e.g. sensitization and habituation due to the repeated stimulus presentations) over the time course of the experiment, the control group was subdivided. Half of the respective participants (N_{2A} = 8) received the same auditory cues (sound A and sound B) as the test group. These acoustic stimuli were however randomly presented with the tepid or cold-water immersions (i.e. truly random control). To ensure counterbalancing of the sounds, the order A B was presented to half of these N_{2A} participants (N_{2Aa} = 4), whereas the other half (N_{2Ab} = 4) perceived the order B A. The first six acoustic stimuli were unpaired with tepid water, the second six ones were paired with ice water immersions. The second half of the control group (N_{2B} = 8) was exposed to the same sequence of tonic stimuli as all the other participants, without however receiving any acoustic cues (see Fig. 1A).

The post-conditioning phase started 15 min after the end of the conditioning phase in order to avoid HNCS-related inhibitory hangover [24]. The purpose of the post-conditioning phase was to investigate associative learning effects that were acquired during the conditioning phase. Before the actual start of the post-conditioning phase, a final BL measurement (BL3) was performed to assess pain intensity, pain unpleasantness and physiological parameters while administering only electrical stimulation. In six post-conditioning test trials, phasic electrical stimuli were simultaneously presented with auditory cues. The counterbalanced order of the auditory cues was the same for the test group and the control group. To avoid possible contamination effects from the CS+ onto the CS– [24], CS– was presented in the first three trials and CS+ in the last three trials (cf. Fig. 1A). The experimental session ended with the removal of all electrodes and verbal debriefing of the participants.

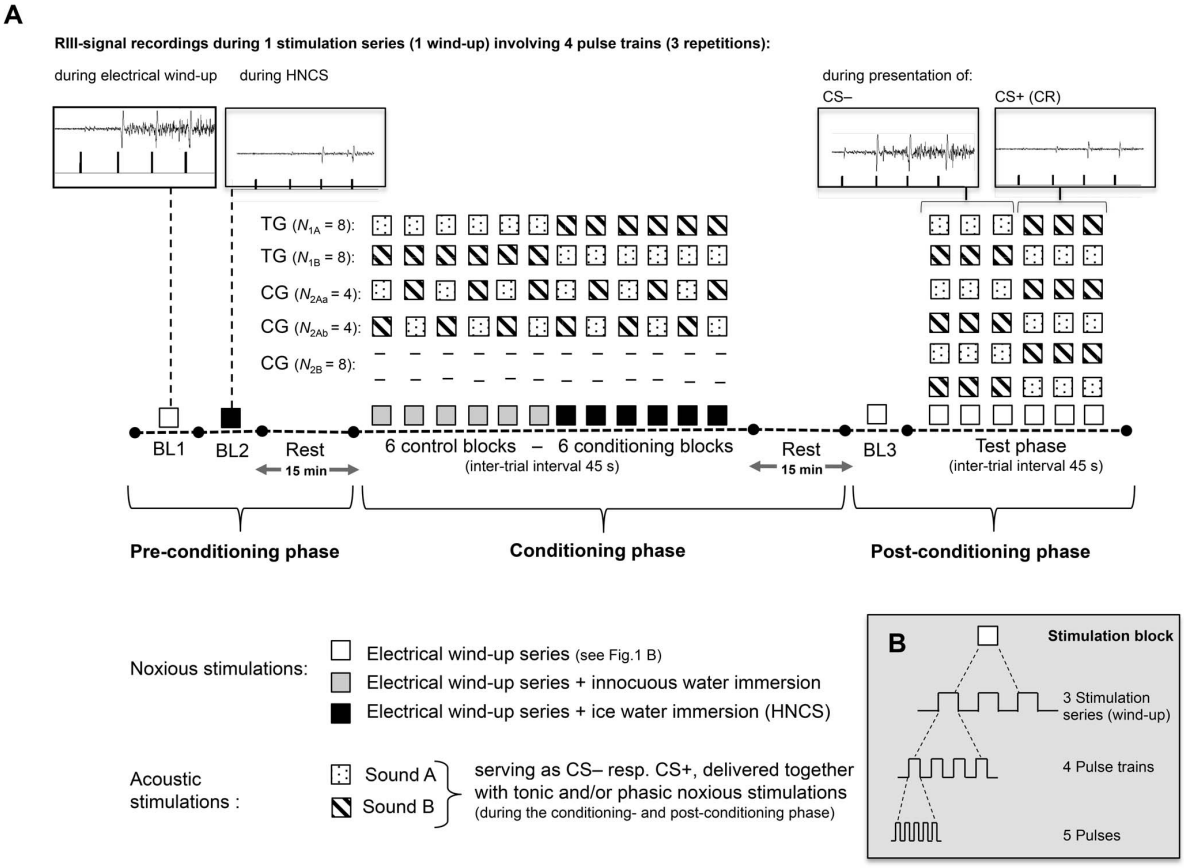

Figure 1: Experimental protocol. Abbreviations: HNCS = heterotopic noxious counter-stimulation, UCR = unconditioned response, CS = conditioned stimulus, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus, CR = conditioned response, TG = test group (N_1 = 16), CG = control group (N_2 = 16), BL = baseline. (A) Stimulus presentations during the pre-conditioning, conditioning and post-conditioning phases (see further details in the text. (B) Electrical stimulation sequences delivered over each stimulation block.

Data analyses

Pain intensity, pain unpleasantness, corrugator and RIII reflex activity were analyzed in response to electrical stimuli. Psychophysical responses were evaluated for the pre-conditioning-, conditioning- and post-conditioning phases. Corrugator and flexion reflex recordings were only examined in association with pre- and post-conditioning trials. To take into account a potential involvement of baroreceptor reflex mechanisms in the regulation of pain sensitivity [37,38], BP and HR data were evaluated in periods including (BL2) and in those not including cold-water immersion (BL1, BL3, CS–/CS+ trials). Possible changes in electrical stimulation conditions throughout the experiment were monitored by contrasting electrodermal activity (EDA; in μS) measured during pre-conditioning BL1 and post-conditioning BL3.

AcqKnowledge Software package (BIOPAC Systems Inc., USA) was used for physiological data collection and offline analysis. To assess the corrugator- and RIII reflex activity, integrated EMG was derived from the respective raw data. For analyses of corrugator muscle activity, the EMG data recorded during each ISI (500 ms between two pulse trains) [39] were used and averaged over each stimulation block. The investigation of overall magnitudes of the RIII reflex responses, as well as RIII wind-up ratios was based on the EMG recording periods ranging from 90 to 180 ms following each pulse train ([40,41,29]; see specimen RIII waveforms in Fig. 1A). To define the overall RIII magnitudes, all EMG-values recorded during each stimulation block were averaged. Wind-up-induced RIII responses were analyzed for each stimulation series by subtracting the reflex amplitudes obtained in response to the first pulse train from those obtained to the last (4^{th}) one. The respective data were then averaged over the 3 stimulation series of each stimulation block and expressed as percent difference (Δ%). Mean (systolic) blood pressure (BP), heart rate (HR) and electrodermal values recorded during the ± 1-min stimulation blocks were analyzed.

HNCS-induced changes in pain ratings and pain-related reflexes were computed by plotting differences between the pre-conditioning BL1 (i.e. phasic pain stimulation only) and BL2 (i.e. phasic pain stimulation + HNCS). The CS– and CS+ values of the test phase trials were averaged to identify differences between the post-conditioning BL3 (i.e. phasic pain stimulation only) measures and the post-conditioning CS– and CS+ (i.e. electrical and acoustic stimulation) values, respectively. HNCS and CS-induced changes in pain and reflex responses were depicted as difference (Δ%).

SPSS software (IBM Corp., USA) was used for the statistical analyses of psychophysical and psychophysiological data. Since some corrugator and RIII values exceeded physiologically reasonable measures (probably related to artefacts like

movement, electrode contact), we decided to consider them as outliers and excluded them from the statistical analysis. The corrugator data of one participant of the test group and of three participants of the control group were left out. Also the RIII values of one participant of the control group could not be included in the statistical analyses. Technical problems with the blood pressure measurement unit resulted in the loss of cardiovascular data from nine participants. Arithmetic mean and standard error values were used as measures for central tendency and dispersion. The normal distribution of the different variables was verified with the Kolmogorov-Smirnov test. Analyses of variance (ANOVA) for repeated measures and post hoc comparisons (parametric *t*-tests for paired samples) were performed to identify significant differences in pain and reflexes between experimental phases. Greenhouse-Geisser corrections were used in case of violation of the sphericity assumption. In addition, we were interested in possible interactions between the group factor (test and control group) and the repeat factor CS– and CS+ in the post-conditioning phase. We computed 2x(2) ANOVA for all dependent variables to uncover potential significant differences between CS– and CS+ values that were characteristic for the test group, but not for the control group. The potential contribution of blood pressure [26] to differences between the test and the control group and gender-related differential expression of RIII reflex responses [42,43] were analyzed by computing between-subject ANOVA and post hoc comparisons (independent *t*-tests). Statistical significance was set at $p \leq 0.05$ (one-tailed).

Results

Psychophysical data

Pain intensity and pain unpleasantness values are represented in Table 1, Table 2, Fig. 2A and Fig. 2B. The Kolmogorov-Smirnov test confirmed a normal distribution of the considered psychophysical variables (all $p > 0.10$).

Pain intensity

The initial cold pressor test performed prior to the experimental protocol induced pain intensities gradually increasing over the ± 1 min stimulation period. Differences between the first and the last (6^{th}) pain intensity ratings on the 10-point NRS ranged in average from 2.6 to 8.1 in the test group and from 2.5 to 7.3 in the control group. Generally, electrical stimulation intensities evoking pain intensity ratings of 3 to 5 corresponded to the RIII reflex threshold.

During BL2 of the pre-conditioning phase, counter-stimulation caused a significant decrease in electrically induced pain intensity in the test group (21% ± 4.1; $t_{15} = 5.78$, $p <$

0.005) and in the control group (15% ± 4.5; $t_{15} = 2.31$, $p < 0.05$) (see Fig. 2A and Table 2). In the post-conditioning (test) phase of the test group, the initially neutral sound that served as paired conditioned stimulus (CS+) was able to inhibit pain (17% ± 4.8). This inhibitory effect was comparable to the one that was found for the HNCS itself (see Fig. 2A). The presentation of the unpaired CS– resulted in a lower pain reduction (8% ± 2.1). Consequently, pain intensity was rated significantly lower during CS+ than during CS– ($t_{15} = 1.94$, $p < 0.05$; see Table 2). In the control group, the presentation of the auditory stimuli did not bring essential alterations in pain intensity (CS+: 1% ± 2; CS–: 1.5% ± 1.6; see Fig. 2A). We did not observe any significant difference between CS– and CS+ presentation ($t_{15} = -.78$, $p > 0.05$) (see Table 2).

Table 1: Psychophysical and psychophysiological data (absolute values)[1]

Outcome measures:	**Pain intensity ratings** NRS (0–10)		**Pain unpleasantness ratings** NRS (0–10)		**Corrugator muscle activity (Frowning reflex)** Integrated EMG (μV)		**Biceps femoris muscle activity (RIII flexion reflex)** Integrated EMG (μV)	
Groups:	**Test group** ($N_1 = 16$) *AM ± SEM*	**Control group** ($N_2 = 16$) *AM ± SEM*	**Test group** ($N_1 = 16$) *AM ± SEM*	**Control group** ($N_2 = 16$) *AM ± SEM*	**Test group** ($N_1 = 15$) *AM ± SEM*	**Control group** ($N_2 = 13$) *AM ± SEM*	**Test group** ($N_1 = 16$) *AM ± SEM*	**Control group** ($N_2 = 15$) *AM ± SEM*
Test stimuli[a]								
Pre-conditioning:								
BL 1	5.4 ± 0.4	5.5 ± 0.4	5.4 ± 0.4	6.3 ± 0.4	0.761 ± 0.10	0.890 ± 0.13	0.138 ± 0.02	0.159 ± 0.02
BL 2	4.3 ± 0.5	4.7 ± 0.4	4.3 ± 0.5	5.3 ± 0.5	0.540 ± 0.06	0.622 ± 0.05	0.116 ± 0.02	0.132 ± 0.02
Conditioning:								
a) Control blocks:								
SB 1	4.4 ± 0.5	5.7 ± 0.5	4.5 ± 0.5	6 ± 0.4				
SB 2	4.7 ± 0.5	5.5 ± 0.5	4.8 ± 0.5	6.2 ± 0.4				
SB 3	4.5 ± 0.5	5.5 ± 0.4	4.7 ± 0.5	6.2 ± 0.4				
SB 4	4.4 ± 0.5	5.1 ± 0.4	4.5 ± 0.5	6.2 ± 0.5				
SB 5	4.4 ± 0.5	5.1 ± 0.4	4.3 ± 0.5	6.1 ± 0.5				
SB 6	4.2 ± 0.5	5.3 ± 0.4	4.2 ± 0.6	6.4 ± 0.4				
b) Acquisition blocks:								
SB 7	2.9 ± 0.5	4.3 ± 0.4	2.9 ± 0.5	5.4 ± 0.5				
SB 8	2.8 ± 0.5	4.4 ± 0.4	3 ± 0.5	5.5 ± 0.5				
SB 9	3 ± 0.6	4.5 ± 0.4	3.1 ± 0.5	5.3 ± 0.6				
SB 10	3.1 ± 0.5	4.5 ± 0.4	3.1 ± 0.5	5.4 ± 0.6				
SB 11	2.9 ± 0.5	4.4 ± 0.4	3.1 ± 0.6	5.3 ± 0.6				
SB 12	2.7 ± 0.5	4.6 ± 0.4	2.8 ± 0.6	5.4 ± 0.6				
Post-conditioning:								
BL 3	4.8 ± 0.4	4.9 ± 0.4	5 ± 0.5	6.4 ± 0.4	0.688 ± 0.10	0.644 ± 0.10	0.125 ± 0.02	0.101 ± 0.01
Test phase:								
CS– 1	4.5 ± 0.5	5.4 ± 0.3	4.5 ± 0.6	6 ± 0.4	0.689 ± 0.10	0.664 ± 0.10	0.123 ± 0.02	0.097 ± 0.01
CS– 2	4.4 ± 0.5	5.3 ± 0.3	4.4 ± 0.5	6 ± 0.5	0.683 ± 0.10	0.618 ± 0.10	0.113 ± 0.02	0.097 ± 0.01
CS– 3	4.5 ± 0.5	5.4 ± 0.4	4.6 ± 0.5	6.2 ± 0.5	0.671 ± 0.10	0.629 ± 0.10	0.116 ± 0.02	0.097 ± 0.01
Mean CS– (1-3)	4.4 ± 0.5	5.4 ± 0.3	4.5 ± 0.5	6.1 ± 0.5	0.681 ± 0.10	0.637 ± 0.00	0.117 ± 0.02	0.097 ± 0.01
CS+ 4	4.2 ± 0.5	5.6 ± 0.4	4 ± 0.6	6.2 ± 0.4	0.652 ± 0.10	0.627 ± 0.10	0.117 ± 0.02	0.090 ± 0.01
CS+ 5	4.1 ± 0.6	5.6 ± 0.4	4.1 ± 0.6	6.2 ± 0.4	0.501 ± 0.07	0.611 ± 0.10	0.111 ± 0.02	0.091 ± 0.01
CS+ 6	3.9 ± 0.5	5.5 ± 0.4	3.8 ± 0.6	6.1 ± 0.4	0.490 ± 0.07	0.558 ± 0.10	0.107 ± 0.02	0.084 ± 0.01
Mean CS+ (4–6)	4 ± 0.5	5.6 ± 0.4	4 ± 0.6	6.2 ± 0.4	0.548 ± 0.10	0.599 ± 0.10	0.112 ± 0.02	0.086 ± 0.01

Pain unpleasantness

Pain unpleasantness was significantly inhibited by HNCS in all experimental groups (24% ± 5.4 for the test group; 18% ± 5.9 for the control group; see Fig. 2B). In addition, we observed a pronounced conditioning effect in the test group exhibiting a pain unpleasantness reduction of 25% (25% ± 6.6) that was typical for CS+, whereas CS– produced significantly less attenuated pain unpleasantness sensations (11% ± 5.6; see Fig.

[1] [a] Phasic electrical stimulation. Abbreviations: NRS = Numerical rating scale, EMG = electromyography, AM = arithmetic mean, SEM = standard error of the mean, BL = Baseline, SB = stimulation block, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus. One-tailed *p*-values of * $p < 0.05$ and ** $p < 0.005$ were considered as significant and highly significant.

2B; $t_{15} = 6.55$, $p < 0.005$; see Table 2). In contrast to this finding, pain unpleasantness ratings in the post-conditioning phase remained almost unaltered under control condition (CS+: 3% ± 2.1; CS–: 3% ± 1.7; see Fig. 2B). The difference between pain ratings related to CS+ and CS– was not significant ($t_{15} = -.65$, $p > 0.05$; see Table 2) in the control group.

The 2x(2) ANOVA analyses of pain rating data did not reveal a significant interaction between the group and the repeat factor CS– and CS+. In these tests, a substantial difference in pain intensity [$F(1,60) = 2.33$, $p > 0.05$] and pain unpleasantness [$F(1,60) = .35$, $p > 0.05$] responses during CS– and CS+ could not be revealed for the test group and for the control group. A significant main effect of group on the sensory-discriminative [$F(1,60) = 6.77$, $p < 0.05$] and the affective-motivational [$F(1,60) = 12.7$, $p < 0.005$] component of pain sensations was observed.

Psychophysiological data

All psychophysiological data are summarized in Tables 1, 2, 3 and 4. Δ % values are shown in Fig. 2C and Fig. 2D. The two examined objective measures are normally distributed (all $p > 0.10$).

Corrugator muscle activity

Counter-stimulation considerably inhibited corrugator muscle activity in all experimental groups (21% ± 5.3 for the test group, 18% ± 4.8 for the control group; see Fig. 2C). In the post-conditioning period, CS+ induced a robust reduction of EMG-activity in the test group (16% ± 4.3; see Fig. 2C). This decline was significantly more pronounced than the one observed under CS– conditions (3% ± 1.6; $t_{14} = 1.71$, $p \leq 0.05$; see Fig. 2C and Table 2). We did not detect any significant CS+ or CS– effect in the control group. The frowning response decreased by 7% ± 2.6 in the CS+ condition and by 1% ± 1.1 in the CS– condition (see Fig. 2C). There were no significant differences in facial expression between these two conditions in this group ($t_{12} = 1.17$, $p > 0.05$; see Table 2).

RIII flexion reflex

Stimulation intensities ranging from 0.1–9.9 mA (3.3 ± 3.01) were required to evoke reliable RIII reflexes.

In the test group, the overall RIII reflex magnitude was reduced by 16% ± 6.1 when electrical stimuli and HNCS were applied concurrently (see Fig. 2D). Under the same conditions, the control group also displayed a reduction in RIII activity of 16% ± 4.8; see Fig. 2D). In the post-conditioning phase, the presentation of CS+ induced reductions of reflex activity of 10% ± 4.8 in the test group and of 11% ± 4.5 in the control group. Reflex attenuations of 8% ± 3.9 in response to the CS– were observed in the test group and of 3%

± 3.6 in the control group (see Fig. 2D). CS– and CS+ induced reflex responses were not significantly different (t_{15} = 1.3, $p > 0.05$; see Table 2) in the test group. In the control group, CS+ was however accompanied by a significantly more pronounced attenuation of the reflex as compared to CS– ($t_{14} = 2.6, p < 0.05$; see Table 2).

In the test group the analysis of the RIII-related wind-up effects throughout all baseline- and test phase trials revealed average increases ranging from 24% to 50% when comparing the 1st and the 4th pulse train of a stimulation series. In the control group, wind-up related increases in reflex activity were realized in all the stimulation blocks of interest and ranged from 28% to 76%. Absolute values related to the 1st and the 4th pulse train, as well as Δ % measures are shown in Table 4.

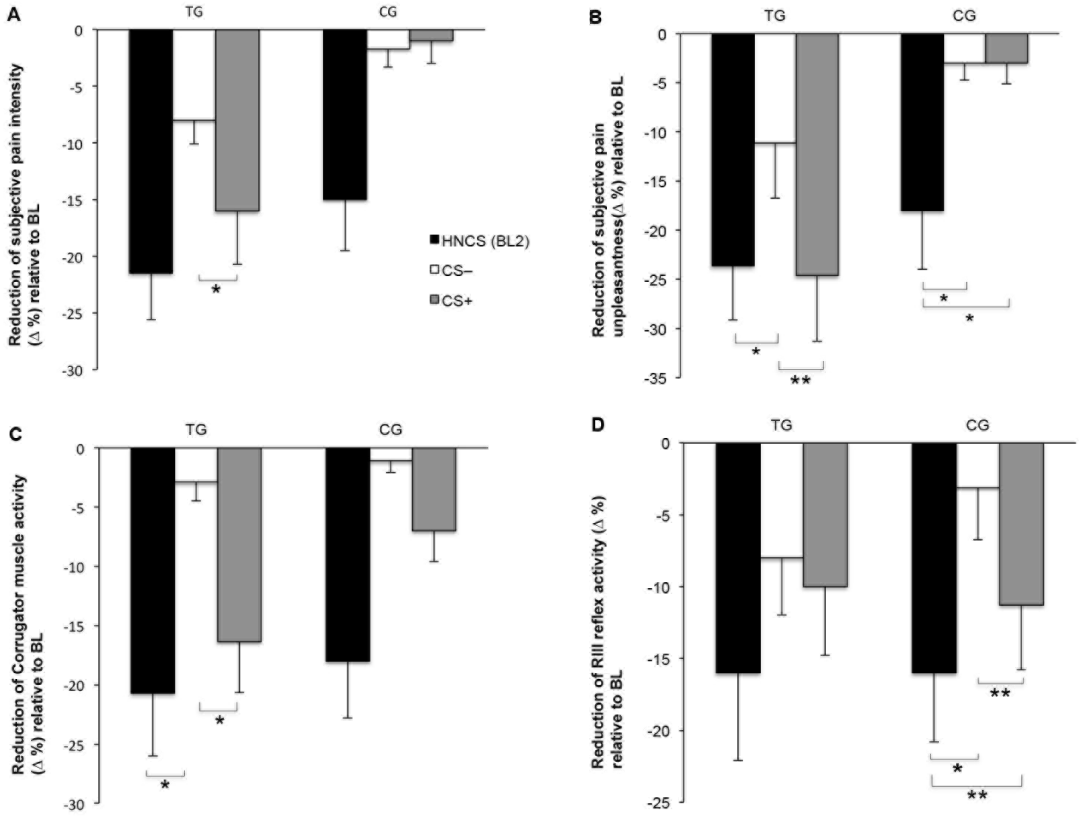

Figure 2: Psychophysical and psychophysiological data of the test group (N_1 = 16) and the control group (N_2 = 16) during pre-conditioning HNCS (BL2) and post-conditioning CS–/CS+ trials (3 CS– trials; 3 CS+ trials). Pre-conditioning BL2 values were contrasted to pre-conditioning BL1 values (1 trial for each BL). Post-conditioning CS–/CS+ values were contrasted to post-conditioning BL3 values. (A) Pain intensity decrease relative to BL. (B) Reduction in pain unpleasantness relative to BL. (C) Inhibition of corrugator muscle activity relative to BL. (D) Overall magnitude RIII reflex inhibition relative to BL.

Abbreviations: TG = test group, CG = control group, HNCS = heterotopic noxious counter-stimulation, BL = baseline, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus, Δ % = percent difference.

Results were based on absolute values and were presented as percent difference measures. Arithmetic mean and standard error of the mean (*AM* ± *SEM*) were used as measures for central tendency and variability. For the differences between test phase effects, p-values of * $p < 0.05$ and ** $p < 0.005$ were considered as significant and highly significant.

No sex-related differences in RIII reduction were observed in our sample (all $p > 0.10$).

When comparing the test and the control group with respect to the CS– and CS+ related corrugator and RIII responses of the post-conditioning phase, the 2x(2) analyses of variance did not disclose any significant interaction for corrugator [$F(1,52) = .33, p > 0.05$] and RIII measures [$F(1,58) = .03, p > 0.05$]. A group main effect on the frowning [$F(1,52) = .002, p > 0.05$] and withdrawal [$F(1,58) = 2.15, p > 0.05$] reflex could not be identified.

Table 2: Psychophysical and psychophysiological statistical overall magnitude data analyses[2]

Baseline – CS comparisons: Pre-conditioning phase: BL1, BL2; Post-conditioning phase: BL3, Test phase: Mean CS–, Mean CS+								
Pain intensity ratings NRS (0–10)	**Within-Subjects Effects:** Main effect 'Pain intensity' (Greenhouse-Geisser correction)			**Post hoc comparisons:**				
				Stimulation block:	**Test group** ($N_1 = 16$)		**Control group** ($N_2 = 16$)	
	F (df)	*p* (one-tailed)	*d*		*t (df)*	*p* (one-tailed)	*t (df)*	*p* (one-tailed)
	4.35 (1.99)	.01*	.13	BL1 – BL2	5.78 (15)	< .005**	2.31 (15)	.01*
				BL3 – mean CS–	1.23 (15)	.12	–.24 (15)	.46
				BL3 – mean CS+	2.67 (15)	.009*	–.60 (15)	.28
				Mean CS– – mean CS+	1.94 (15)	.03*	–1.46 (15)	.08
Pain unpleasantness ratings NRS (0–10)	**Within-Subjects Effects:** Main effect 'Pain unpleasantness' (Greenhouse-Geisser correction)			**Post hoc comparisons:**				
				Stimulation block:	**Test group** ($N_1 = 16$)		**Control group** ($N_2 = 16$)	
	F (df)	*p* (one-tailed)	*d*		*t (df)*	*p* (one-tailed)	*t (df)*	*p* (one-tailed)
	6.87 (2.23)	< .005**	.18	BL1 – BL2	4.59 (15)	< .005**	2.53 (15)	.01*
				BL3 – mean CS–	–1.54 (15)	.07	.74 (15)	.23
				BL3 – mean CS+	3.39 (15)	< .005**	–.06 (15)	.47
				Mean CS– – mean CS+	6.55 (15)	< .005**	–.65 (15)	.26
Corrugator muscle activity (Frowning reflex) Integrated EMG (μV)	**Within-Subjects Effects:** Main effect 'Corrugator' (Greenhouse-Geisser correction)			**Post hoc comparisons:**				
				Stimulation block:	**Test group** ($N_1 = 15$)		**Control group** ($N_2 = 13$)	
	F (df)	*p* (one-tailed)	*d*		*t (df)*	*p* (one-tailed)	*t (df)*	*p* (one-tailed)
	3.33 (2.13)	.02*	.11	BL1 – BL2	3.05 (14)	< .005**	2.08 (12)	.03*
				BL3 – mean CS–	.28 (14)	.39	.27 (12)	.39
				BL3 – mean CS+	1.68 (14)	.05*	.89 (12)	.19
				Mean CS– – mean CS+	1.71 (14)	.05*	1.17 (12)	.13
Biceps femoris muscle activity (RIII flexion reflex) Integrated EMG (μV)	**Within-Subjects Effects:** Main effect 'RIII' (Greenhouse-Geisser correction)			**Post hoc comparisons:**				
				Stimulation block:	**Test group** ($N_1 = 16$)		**Control group** ($N_2 = 15$)	
	F (df)	*p* (one-tailed)	*d*		*t (df)*	*p* (one-tailed)	*t (df)*	*p* (one-tailed)
	9.80 (1.55)	< .005**	.25	BL1 – BL2	2.35 (15)	.02*	3.23 (14)	< .005**
				BL3 – mean CS–	1.33 (15)	.10	1.44 (14)	.08
				BL3 – mean CS+	1.69 (15)	.05*	2.95 (14)	≤.005**
				Mean CS– – mean CS+	1.33 (15)	.10	2.63 (14)	.01*

Blood pressure, heart rate and electrodermal activity

The test group and the control group did not significantly differ with regard to BP values (see Table 3; $p > 0.05$). In both groups, blood pressure increases were only observed during BL2 of the pre-conditioning phase, where cold-water stimulation (HNCS) generated average BP increases ranging from 25 to 56 mmHg. The differences between BP values recorded during stimulation blocks without cold water immersion (baseline- and CS-related phases) and the one with ice-water immersion (BL2) were all significant (all $p < 0.05$; see Table 3)

In both groups, mean HR values varied between 93 and 96.5 beats per minute (BPM) and remained stable throughout all the experimental phases. Analyses of EDA responses

[2] Abbreviations: CS = conditioned stimulus, BL = Baseline, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus, NRS = Numerical rating scale, EMG = electromyography, SB = stimulation block. One-tailed *p*-values of * $p < 0.05$ and ** $p < 0.005$ were considered as significant and highly significant.

did not reveal any significant difference between pre- (BL1) and post-conditioning BL (BL3; $\Delta < 200\ \mu S$ in all groups).

Table 3. Systolic blood pressure measures (mmgH)[3]

	Test group ($N_1 = 11$)	Control group ($N_2 = 12$)	Between-Groups Effects:		Post hoc comparisons Test group – Control group:	
	AM ± SEM	AM ± SEM	*F (df)*	*p* (one-tailed)	*t (df)*	*p* (one-tailed)
Pre-conditioning phase:						
BL 1	13.3 ± .55	13.5 ± .64	.05 (1)	40	–.23 (21)	.40
BL 2	15.4 ± .91	15.4 ± .66	.00 (1)	.49	–.01 (21)	.49
Post-conditioning phase:						
BL 3	13.4 ± .41	12.6 ± .52	1.42 (1)	.12	1.19 (21)	.12
CS– 1	13.3 ± .33	12.8 ± .55	.57 (1)	.22	.75 (21)	.22
CS– 2	13.0 ± .44	12.7 ± .58	.16 (1)	.34	.40 (20)	.34
CS– 3	13.2 ± .47	12.4 ± .53	1.29 (1)	.13	1.13 (21)	.13
CS+ 1	13.2 ± .39	12.6 ± .54	1.00 (1)	.16	1.00 (21)	.16
CS+ 2	13.4 ± .37	12.7 ± .54	.00 (1)	.47	–.06 (21)	.47
CS+ 3	12.9 ± .50	13.0 ± .54	.03 (1)	.42	.18 (21)	.42
Mean CS– (1–3)	13.0 ± .42	12.8 ± .55	.71 (1)	.20	.84 (21)	.20
Mean CS+ (4–6)	13.1 ± .39	12.8 ± 52	.14 (1)	.35	.37 (21)	.35

Table 4: RIII reflex-related wind-up values[4]

Biceps femoris muscle activity (RIII flexion reflex) - **RIII wind-up** measures - Integrated EMG (μV)						
	Test group ($N_1 = 16$) *AM ± SEM*			Control group ($N_2 = 15$) *AM ± SEM*		
	1st pulse	4th pulse	Wind-up effect (Δ%)	1st pulse	4th pulse	Wind-up effect (Δ%)
Test stimuli[a]						
Pre-conditioning:						
BL 1	0.028 ± 0.006	0.041 ± 0.006	43 Δ%	0.029 ± 0.005	0.050 ± 0.009	72 Δ%
BL 2	0.024 ± 0.003	0.036 ± 0.007	50 Δ%	0.026 ± 0.003	0.039 ± 0.006	53 Δ%
Post-conditioning:						
BL 3	0.024 ± 0.004	0.035 ± 0.006	46 Δ%	0.020 ± 0.002	0.025 ± 0.003	28 Δ%
Test phase:						
CS– 1	0.028 ± 0.006	0.037 ± 0.007	34 Δ%	0.020 ± 0.002	0.025 ± 0.002	26 Δ%
CS– 2	0.027 ± 0.006	0.035 ± 0.006	29 Δ%	0.017 ± 0.001	0.028 ± 0.003	60 Δ%
CS– 3	0.030 ± 0.007	0.035 ± 0.006	17 Δ%	0.020 ± 0.003	0.025 ± 0.003	23 Δ%
Mean CS– (1-3)	0.028 ± 0.006	0.036 ± 0.006	27 Δ%	0.019 ± 0.002	0.026 ± 0.003	35 Δ%
CS+ 4	0.029 ± 0.007	0.038 ± 0.007	30 Δ%	0.018 ± 0.002	0.024 ± 0.003	35 Δ%
CS+ 5	0.028 ± 0.007	0.036 ± 0.006	25 Δ%	0.018 ± 0.002	0.026 ± 0.003	40 Δ%
CS+ 6	0.027 ± 0.006	0.031 ± 0.005	16 Δ%	0.018 ± 0.002	0.023 ± 0.003	24 Δ%
Mean CS+ (4–6)	0.028 ± 0.007	0.035 ± 0.006	24 Δ%	0.018 ± 0.002	0.024 ± 0.003	33 Δ%

[3] Abbreviations: AM = arithmetic mean, SEM = standard error of the mean, *df* = degrees of freedom, BL = baseline, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus. One-tailed *p*-values of * $p < 0.05$ and ** $p < 0.005$ were considered as significant and highly significant.

[4] One stimulation block included three phasic electrical stimulation series (wind-up). Each stimulation series comprised four electrical pulse trains (see Fig. 1B). The wind-up effects were calculated by substracting reflex-induced EMG-values obtained in response to the first pulse train from those obtained to the last (4th) one. The respective data were then averaged over the three stimulation series of each stimulation block and presented in percent difference (Δ%).

Abbreviations: EMG = electromyography, AM = arithmetic mean, SEM = standard error of the mean, BL = Baseline, CS– = unpaired conditioned stimulus, CS+ = paired conditioned stimulus.

Discussion

To our knowledge, this is the first study to uncover that differential Pavlovian (i.e. respondent) conditioning is able to activate endogenous 'pain inhibits pain'-like mechanisms. Associative learning processes thus seem to have the capacity to sustain HNCS-induced hypoalgesia. Our results do indeed show that after repeatedly associating a tonic noxious stimulus (i.e. cold water bath, HNCS) with a differential acoustic stimulation, the paired auditory cue (CS+) was able to attenuate the electrically induced pain sensations in the test group. This decrease in pain sensitivity was accompanied by a reduction of corrugator supercilious muscle activity. This finding is reminiscent of a previous work by Flor and co-workers [15] describing successful classical conditioning of stress-induced analgesia and inherent opioid release.

Recent imaging studies have shown that in addition to classically described spinal cord-brainstem loops, brain areas like the ACC and the amygdala are also involved in pain modulation evoked by HNCS [11,12]. Consequently, the implication of these brain structures in both learning [44,45,46,14] and pain modulation processes corroborates the hypothesized relationship between the endogenous pain control systems and associative learning of cues from an individual's environment. Traditionally, learning processes have been claimed to be involved in the development and maintenance of pain and of pain-related behavior (for review see [16]). In contrast, the present study is devoted to the potential impact of learning on pain inhibition and hence on the potential usefulness of conditioning procedures for the treatment of pain states. Whereas Flor and co-workers [15] investigated the influence of learning on stress-induced analgesia, we focused on HNCS-activated inhibition of nociceptive processing which proved to be a handy tool to assess endogenous pain control systems in both experimental [4,5,6] and clinical [7,8] settings. The strong learning effects that we identified point to a potential relevance for the development of novel psychological treatment strategies. Further support for this notion may be derived from persisting effects of stimulation procedures like acupuncture or TENS. The positive therapeutic effect of these techniques has been discussed to result from DNIC-like processes [17]. But since DNIC generally last for periods of several minutes [10], associative learning effects probably partially mediate the repeatedly proven, long-lasting therapeutic efficacy of acupuncture and TENS [19,20].

With respect to the conditioning paradigm, it should be noted that the concurrent initiation and duration of the CS and the US might be indicative of a simultaneous conditioning procedure. It should however be taken into account that the ice water-related pain sensations typically occurred after an immersion period of about 20 seconds [36]. In fact, the onset of the CS thus preceded the HNCS-related effects by this time interval.

Consequently, the learning procedure may rather be considered as delay conditioning (for review see [47]). This paradigm is commonly used as an effective tool for the conditioning of emotional reactions and requires brain regions like the ones mentioned above. It is characterized by a reduced participation of hippocampal activity which may rather encode temporal information related to time intervals passing between CS and US onset that are characteristic of trace conditioning [48].

In the present study, physiological indicators of nociceptive processing were included in addition to psychophysical parameters. In particular, we measured noxious stimulation-induced reflexes of the corrugator (frowning or brow lowering reflex) and biceps femoris muscle (RIII flexion reflex). We decided to measure corrugator activity since changes in frowning activity have been shown to be a reliable tool to assess non-verbal pain expression [2,39,5], with a major emphasis on the affective dimension of pain (e.g. pain unpleasantness. In this context, emotional expression (e.g. pain-related facial expression in social settings) has been shown to determine frowning reflex amplitudes [49]. These findings could account for the observed respondent conditioning effect of the frowning response. In fact, the magnitude of the facial motor behavior inhibition upon presentation of CS+ in the post-conditioning phase was comparable to the one recorded during the HNCS procedure.

Since the nocifensive RIII flexion reflex has repeatedly been assessed in studies on the DNIC phenomenon [40,24,50], we included it as a second objective indicator of nociceptive processing. In line with the cited previous findings, we also found a reduction of RIII-related EMG activity upon HNCS. In response to the post-conditioning CS+ presentation however, attenuations of withdrawal reflex responses were observed both in the test group and in the control group. Furthermore, the CS– induced reductions in the RIII reflex activity of the test group were quite similar to those provoked by CS+ stimulations. We can thus conclude that, contrary to our initial hypothesis, respondent conditioning had no significant influence on the RIII flexion reflex. The successful conditioning of pain perception and corrugator muscle activation and the lack of conditioning effects on RIII reflex amplitudes observed in the present study may be related to differential neural circuitry involved in the respective reactions. The nocifensive RIII reflex is known to depend mainly on segmental spinal processing to ensure rapid and reliable withdrawal from noxious stimuli [23]. Accordingly, it was found to be unchanged in paraplegic patients [51]. Corrugator muscle activity and pain sensitivity are more significantly governed by higher order brain structures like prefrontal and cingulate cortical areas and the amygdala [52,53], which are also heavily involved in learning processes and

in emotional regulation. It thus seems plausible that these structures provided a more suitable substrate for Pavlovian conditioning.

We measured BP and HR to control for potential confounding effects of baroreceptor reflex-mediated modulation of pain sensitivity triggered by repeated immersion of the hand into cold water [37,38]. It could be observed that the blood pressure only went up during BL2, when ice-water stimulation was paired with electrical stimuli. In all the other phases and throughout the groups, it did not vary notably. The described conditioning of pain inhibition is thus not likely to be attributable to alterations in cardiovascular reactivity. We also chose to apply the constant voltage paradigm in order to provide stable electrical stimulation conditions [27,28]. This stability was confirmed by the fact that EDA and required stimulation intensity levels remained unaltered throughout the experiment.

The conditioning effects observed in the present study could theoretically also be explained by habituation effects related to the long stimulation sequences and by a concomitant reduction in pain ratings and measures. It should however be noted that during the CS+ stimulations in the test phase, pain and corrugator muscle activity did only decrease in the test group and not in the control group that was exposed to the same number of stimuli. Moreover, no habituation time course (1/e function) of the dependent variables of interest was revealed, neither in the test group nor in the control group. As concerns potential distraction effects, the CS+ may be claimed to signal impending pain and therefore to be associated with distracting negative emotions during noxious stimulus presentation. It has been shown in this regard that negative emotions generated during unpredictable noxious (electrical) stimulations imply increases in subjective pain ratings and in absolute RIII reflex magnitudes, whereas predictable noxious stimuli induce an increase in pain sensations and a consistency in RIII reflex magnitude [54]. Our results however exhibit CS+ induced reductions in subjective ratings and decreases in RIII reflex values, which corroborate the lack of involvement of distracting emotions. It has in addition been shown that distraction does not affect RIII reflex activity induced by electrical stimulation sequences allowing for temporal summation to build up [32]. In the present study, though, the RIII reflex activity was reduced following CS+ administration while a wind-up effect was consistently realized in all stimulation blocks.

Despite our inability to provide significant data with respect to CS and group related interactions that may be due to the relatively small sample sizes, the present experimental study still provides new psychophysical and physiological evidence for the involvement of learning effects in endogenous pain control. Since our findings may be relevant for the clinical setting, further studies need to be conducted to determine the prerequisites of respondent conditioning-induced pain attenuations. Additionally, future research will have

to investigate the persistence of these effects. It should finally be mentioned in the present framework that specific learning conditions could also lead to attenuated activity of endogenous pain control pathways and hence be involved in the exacerbation and chronification of pain states. Further research activities will have to be devoted to this important issue.

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3.2 Study 2: Rumination and interoceptive accuracy predict the occurrence of the thermal grill illusion of pain

Abstract

Background: While the neurophysiological mechanisms underlying the thermal grill illusion of pain (TGI) have been thoroughly studied, psychological determinants largely remain unknown. The present study aimed to investigate whether cognitive and affective personality traits encompassing rumination, interoception, and suggestibility may be identified as characteristics favouring the elicitation of paradoxical pain experiences.

Methods: The dominant hand of 54 healthy volunteers was stimulated with a water-bath driven thermal grill providing an interlaced temperature combination of 15 and 41°C. Pain intensity and pain unpleasantness perceptions were rated on a combined verbal-numerical scale (NRS). Traits were assessed via questionnaires, the heartbeat-tracking task, and warmth suggestions.

Results: Logistic regression analyses uncovered trait rumination and interoceptive accuracy (IA) as major predictors of the likelihood of the illusive pain occurrence (all $p <$ .05). Rumination and suggestibility had an impact on unpleasant pain perceptions.

Conclusion: Our findings allowed to identify psychological factors substantially involved in the individual pre-disposition to reporting painful sensations in the thermal grill paradigm. These psychological characteristics may also be relevant in the context of central neuropathic pain, which to a large extent incorporates the same neural pathways.

Rumination and interoceptive accuracy predict the occurrence of the thermal grill illusion of pain

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Introduction

Thermal grill illusion of pain

Since Thurnberg revealed in 1896 that interlaced and non-noxious cold and warm stimuli applied to the skin generate the thermal grill illusion of pain (TGI), a paradoxical feeling of pain, the underlying neurophysiological mechanisms have thoroughly been studied (Craig and Bushnell 1994; Craig et al. 1996, 2000; Kern et al. 2008; Lindstedt et al. 2011b). Functional imaging studies on the TGI have uncovered an involvement of cerebral structures like the contralateral thalamus (Lindstedt et al. 2011b), the anterior cingulate cortex (Craig et al. 1996), and the insula (Craig et al. 2000) that are to a large extent also involved in the regulation of emotions and of interoceptive awareness (Craig 2002). Since the identified neuroanatomical substrates suggest that the illusive pain might share common mechanisms with central neuropathic pain, the thermal grill has been used as a model for the investigation of central pain-related neural activity (Craig 2008).

Inter-individual differences in thermal grill responsiveness

A number of studies have provided evidence for inter-individual differences in thermal grill-related pain sensitivity (Boettger et al. 2011; Bouhassira et al. 2005, Lindstedt et al. 2011a). It could be shown that painful sensations in response to thermal grill stimulation were only perceived by about one third of the participants. Those individuals were qualified as responders to the TGI, whereas those who reported non-painful warm or/and cold sensations or very low pain were described as non- or poor-responders (Boettger et al. 2013; Bouhassira et al. 2005). The reasons for the observed inter-individual differences in TGI susceptibility remain unknown to this point.

We hypothesized that the described differences in susceptibility to the expression of pain could at least partly be related to psychological features. The identification of the previously mentioned cortical areas involved in the TGI as well as in emotional regulation (Craig 2002) seems to underpin this assumption. Further support may be derived from the multidimensional character of pain (Wiech and Tracey 2009) implying that psychological factors are heavily involved in the regulation of pain sensitivity in different pain conditions or experimental pain models. It could in particular be shown that affective and cognitive characteristics promote discrepancies between induced and perceived pain intensity levels (Pennebaker 1999; Wiech and Tracey 2009). Subjects with high levels of anxiety or attention to pain did e.g. display more pronounced ratings to noxious stimulation than

people exhibiting lower values of the mentioned psychological characteristics (Tang and Gibson 2005).

So far however, investigations on the impact of psychological features on the manifestation of paradoxical pain responses remain very scarce. Only the pain enhancing effects of depression and sad mood on thermal grill-activated central pain processing have been confirmed in clinical studies (Boettger et al. 2011; Piñerua-Shuhaibar et al. 2011).

Personality traits and pain

In this framework, we turned towards personality traits that have been identified as important pain modulating factors in classical pain research (i.e. under conditions of evident noxious stimulation). Psychological characteristics such as pessimism, pain catastrophizing, anxiety and related negative affectivity (Crombez et al. 1998; Sullivan et al. 2001a; Affleck et al. 2001), maladaptive coping styles (Keefe et al. 1989; Smith and Alloy, 2009) or biased cognitive processes (Crombez et al. 2013) have repeatedly been described to be associated with increased pain perceptions or pain distortions (Crombez et al. 1998; Edwards et al. 2006; James et al. 2002; Sullivan et al. 2001a, 2005; Tang and Gibson, 2005; Wiech and Tracey, 2009).

Trait pessimism versus trait optimism

Experimental (Affleck et al. 2001, Geers et al. 2008; Mahler and Kulik 2000) and clinical (Goodin et al. 2013) findings suggest that pessimistic individuals feel more pain than optimistic pain patients or healthy volunteers. It has been claimed that pessimistic persons turn more attention to pain, have negative expectations concerning future outcomes, are rather convinced of their inability to deal with problems, and refer to maladaptive coping methods (Geers et al. 2008). Optimists in contrast are more likely to expect favorable outcomes and relate to positive cognitions and behaviours to adjust to or disengage from negative or painful experiences (i.e. approach coping style; Goodin et al. 2013). Hanssen and coworkers (2013) have shown that the relationship between optimism and low pain intensity ratings is mediated by low pain catastrophizing.

Trait pain catastrophizing, trait anxiety, and trait rumination

It has been observed that high trait pain catastrophizing is concomitant with increased anxiety, attention to and anticipation of pain and enhances painful sensations (Crombez et al. 1998; Edwards et al. 2006; Keefe et al. 1989; Sullivan et al. 2001a, 2005, Van Damme

et al. 2004). There also exists a relationship between high trait anxiety and increased pain intensity resp. state anxiety (Ploghaus et al. 2001; Tang and Gibson 2005). The inability to repress pain-related feelings and thoughts constitutes a major stressor for catastrophizing and anxious persons and strongly promotes ruminative thinking (Edwards et al. 2006). Trait rumination is characterized by perseverative thinking on negative events and a deficient cognitive control of ongoing thoughts and is considered as a dimension of the pain catastrophizing construct [cf. Pain Catastrophizing Scale (PCS), Sullivan et al. 1995]. In high ruminators, goal-directed and problem-based coping is hampered by adverse expectations and difficulties in accepting upsetting episodes or in deflecting their attention from problems and bad feelings (Smith and Alloy 2009).

Expectations and suggestibility

Pain magnitude and pain unpleasantness have been reported to depend on the intensity of expected pain (Atlas and Wagner 2012; Boersma and Linton 2006; Tracey 2010). In placebo-related settings, low expectations have been found to play a pain-reducing role (Price et al. 1999), whereas high pain expectancy promoted a negative response or nocebo effect while being interrelated with more anxiety and worrisome feelings (Benedetti et al. 2007; Sawamoto et al. 2000). Another psychological characteristic closely linked to positive and negative pain-related placebo effects is suggestibility (De Pascalis et al. 2002; Staats et al. 1998). It is widely accepted that pain may be lowered in highly suggestible participants following a suggestion of an efficient pain-relieving drug (De Pascalis et al. 2002) or be increased following nocebo stipulations (Staats et al. 1998).

Interoceptive accuracy

The psychophysiological feature interoceptive accuracy (IA) was considered as an additional potential predictor of pain responses to the thermal grill application. The ability to discern internal bodily states is regarded as a stable trait (Tsakiris et al. 2011) and has been highly associated with a tendency of experiencing more intense emotions (Wiens et al. 2000) and of being inclined to more anxiety and catastrophizing (Critchley et al. 2004; Pollatos et al. 2007). This proneness to stronger emotional feelings can lead to a dysfunctional cognitive processing of interoceptive states and to a misjudgement of bodily signals (Wölk et al. 2013). As a consequence, the experience of somatic symptoms is enhanced (Critchley et al. 2004) or over-reported (Barsky and Borus 1999). Biased emotional decision-making (Garfinkel and Critchley 2013; Sütterlin et al. 2013; Wölk, et al. 2013) and an expectation of possibly negative consequences have also been shown in individuals scoring high in interoceptive accuracy. Interestingly, in research based on

suprathreshold noxious stimulation, Pollatos et al. (2012) revealed that participants correctly perceiving their cardiac signals had lower pain threshold and tolerance levels than interoceptively less accurate individuals. Wiech and Tracey (2009) reported that interoception is linked to higher pain perceptions when negative emotional factors like anxiety, catastrophizing, and expectation of pain are involved.

The relationships between pain-related emotional and cognitive personality traits and pain perceptions described in the present study have been derived from classical pain research where they explain inter-individual differences in pain responsiveness to noxious experimental stimulation or to pathological pain conditions. We hypothesized that these psychological and psychophysiological features might not only be involved in the quantitative modulation of pain responsiveness, but also in the qualitative crossover from non-painful to painful sensations in the absence of peripheral noxious input. An identification of dispositional feelings and thoughts affecting thermal grill perceptions was expected to improve the understanding of differential paradoxical pain sensitivity and potentially to provide additional insight into the processes influencing central neuropathic pain syndromes. To test our hypothesis, we first identified responders and non-responders to the thermal grill stimulation by means of subjective ratings of thermal grill-related pain intensity and pain unpleasantness (Boettger et al. 2011, 2013; Bouhassira et al. 2005). In a further step, the personality features trait pessimism–optimism, trait pain catastrophizing, trait anxiety, trait rumination, expectancy of pain, suggestibility, and IA were individually assessed in the participants to characterize responders and non-responders to the TGI and to provide evidence by means of logistic regression analyses that volunteers displaying high levels of specific pain-related traits are more likely to feel the TGI.

Methods

Participants

A sample of 66 healthy participants comprising student and staff populations of the University of Luxembourg was screened. Health-related issues were retrieved with a medical history questionnaire. Depression or mood problems were in addition appraised on the basis of the self-report trait and state questionnaires. Only volunteers without psychological-, cardiovascular-, neurological-, pain-, and skin-related disorders or problems were included in the study. Drugs and pain medication intake 24 hours before experimental testing were also considered as exclusion criteria. Prior to the experimental session, participants were informed that the study was about investigating potential differences in temperature-related perceptions. Furthermore, the volunteers were briefed

about the anonymization of the obtained data and their right of withdrawal without any further consequences. All participating volunteers gave informed consent. The true scientific rationale of the study was provided in the debriefing at the end of the laboratory session. The experimental protocol was approved by the National Research Ethics Committee (ref. 1102–59) and complied with the ethical guidelines of the International Association for the Study of Pain (IASP; Charlton, 1995). Ten participants were excluded from the study since they experienced pain in the control conditions i.e. when stimulated with neutral 32°C (normal skin temperature) in combination with either the warm or cold temperature used for the elicitation of the TGI. The 11th 'outlier' could not be included in the final sample due to technical problems with the thermal grill and incomplete pain ratings. The data of one participant displaying depressive symptoms were excluded from the analyses. The final sample included 54 participants [26 males, 28 females, M = 24.1 years (SD = 6.01), range 18–51 years]. All volunteers were financially compensated.

Material

Thermal grill and accessories

A custom-built and water-bath driven thermal grill device was used to elicit the paradoxical pain (Curio, I., PhD, Medical Electronics, Bonn/Germany). The thermal grill was composed of eight alternating cold and warm pipes made of borosilicate glass. The glass pipes were spaced at a distance of 7.5 mm by means of separating bars to prevent any 'mixing phenomenon' between pipes. The bars were made of 5 mm hollow (thickness 0.5 mm) polyvinyl chloride (PVC) with negligible thermal conductivity. The total surface of the rectangular pipes measured 20 × 10 cm (see Figure 1). The temperatures were regulated with two separate thermoelectric recirculating chillers (T255P, ThermoTek Inc.) delivering the water to the grill pipes through separate flexible and insulated plastic conduits. The flow rate of the pump was 3,86 l/min, approx. 15 ml/s per glass pipe. The volume of one glass pipe was about 16.5 cm^3. The fluid content of each pipe was exchanged at a rate of about one second. The fluid temperature was continuously controlled with a digital thermometer (PL-120 T2, Voltcraft; visual display of T1-T2 temperatures in °C) placed at the manifold, where the water flow was distributed to the glass pipes. Previous measurements have shown that a stationary temperature distribution was reached about 3 s after applying the skin to the pipes.

Figure 1. Custom-built, water-bath driven thermal grill device;
W: warm tubes; C: cold tubes

For the experimental thermal grill condition, we preferred stimulating all participants with the same fixed temperature combination of 15°C and 41°C, instead of individualized temperatures defined in association with previously assessed thermal pain thresholds (as described in studies using Peltier-driven thermal grills; Bouhassira et al. 2005;). This choice was based on the circumstance that water-bath-related temperature changes are time-consuming and on the finding that larger differences between cold and warm grill temperatures allow generating reasonable pain intensities (Boettger et al. 2011; Bouhassira et al. 2005; Lindstedt et al. 2011a). The chosen temperature combination of 15°C and 41°C (difference of 26°C degrees; Boettger et al. 2011; Bouhassira et al. 2005; Lindstedt et al. 2011a) was applied throughout the one-minute trials of the experimental condition (see Figure 2). An inter-stimulus-interval (ISI) of three minutes was always respected between the trials. The same temporal procedure was applied in the two subsequent control conditions. In control condition 1, the cold temperature of 15°C was combined with the baseline temperature of 32°C, whereas in control condition 2 the warm temperature of 41°C was set together with the 32°C input (see Figure 2). As an alternative to previous research procedures using single stimulations (e.g. 15°C in all thermal grill tubes) for control, we preferred providing dual interlaced temperature stimulations mimicking the spacing of the respective temperatures in the experimental 15°C/41°C phase. The order of the stimulation conditions was not counterbalanced to allow for comparability between the responder and non-responder groups.

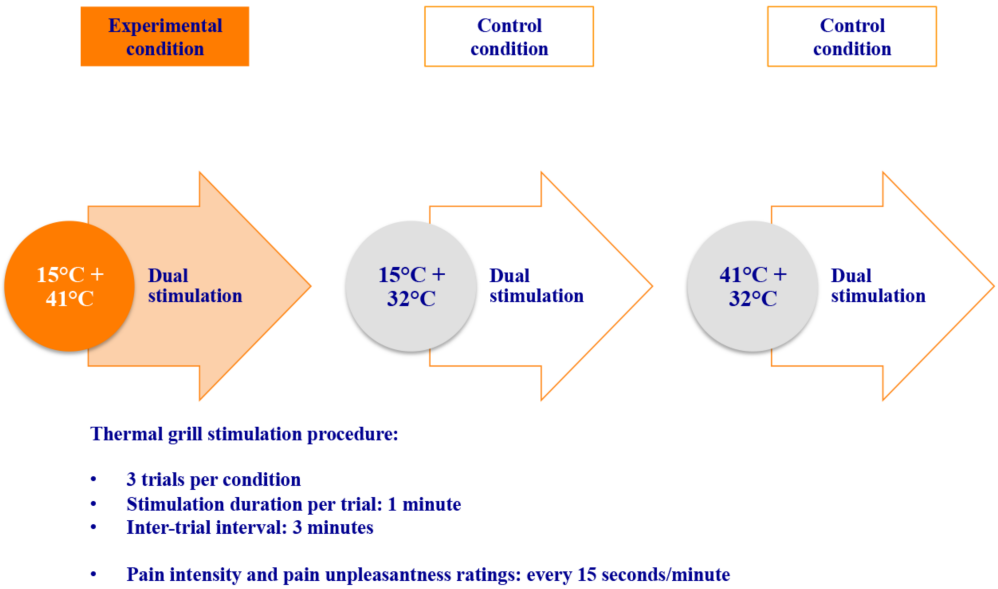

Figure 2: Thermal grill stimulation sequences

The thermal grill stimuli were always applied at the palmar side of the dominant hand. The hand of the participant was placed on the thermal stimulation surface and held in place with a cuff to warrant an equilibrated and integral contact between the hand and the grill bars. The cuff was inflated with a sphygmomanometer (mmHg) until a gentle pressure held the hand in the adequate position. The contact area of the skin to the glass bars (effective surface) was approximately 0.8 cm × 8 (effective glass pipe width in contact with skin × 8 pipes) × 11 cm (width of the hand) = 70.4 cm^2. Applying a pressure of 0.7 MPa (0.071 kp/cm^2 = 50 mmHg), the resulting force was about 0.5 kp. It was considered quite unlikely that the gentle pressure applied with the cuff continuously stimulated the cutaneous mechanoreceptors (which adapt fairly quickly) and influenced the perception of the TGI or changed the suggestibility of the participants. Furthermore, although a modulation of spinal nociceptive processing by concomitant low threshold A-fiber input is well established (Handwerker et al. 1975), this effect was not expected to play a role in the present stimulus conditions, which do not involve any nociceptive input to the dorsal horn that could be modulated. After each stimulation phase, the cuff was detached and the volunteers removed the hand from the grill during the ISI to prevent carry-over effects (Boettger et al. 2011; Bouhassira et al. 2005). Between the different stimulation conditions, a time interval of about 10 minutes had to be respected to allow for adjustment of the targeted grill temperature combination.

Contact heat stimulator

During the so-called generalization suggestion of the Warmth Suggestibility Scale (WSS; Gheorghiu et al. 2003), thermal stimuli of a baseline temperature of 32°C (Morin and Bushnell 1998; Lindstedt et al. 2011a) were applied with a Peltier-driven and temperature controlled contact heat evoked potential (CHEP) stimulator (Pathway, Cheps, Medoc Ltd, Israel) and a thermode with a contact surface of 30x30 mm. Constant warm stimuli of one minute duration were delivered to the non-dominant hand of the participant.

Physiological assessments

The MP150 Data Acquisition System (BIOPAC Systems Inc., USA) was used to record the cardiac activity during the heartbeat-tracking task. Disposable pre-gelled Ag-AgCl electrodes (diameter 35 mm, EL502, Biopac Systems) were placed below the right clavicle and below the left lower rib to perform the standard precordial lead II electrocardiogram (ECG; ECG100C; 0.5 Hz high pass filtering, R-wave output mode, signal gain 500). Subjects were grounded through a similar electrode positioned below the right lower rib. ECG recordings were continuously computed during the heart rate perception measure. Physiological data collection and offline analyses of the frequency of the recorded R-waves were realized with the AcqKnowledge Software package (BIOPAC Systems Inc., USA).

Measures

Pain rating scales

Expectancy of pain was assessed with a visual analogue scale (VAS) measuring 100 mm. The scale was anchored from 0 = *no pain expected* to 100 = *intolerable pain expected*. The intensity of pain participants had expected to feel during the experiment before coming to the lab was assessed at the end of the experimental session to avoid the occurrence of undesirable pain suggestions potentially having an impact on the responses to the subsequently presented sensory stimuli (Arntz and Claassens 2004; Wiech et al. 2008).

Pain intensity and pain unpleasantness ratings. The affective-motivational component of pain was assessed in addition to the sensory-discriminative aspect since both dimensions can vary independently in the sense that emotional characteristics may affect pain unpleasantness sensations without however changing the sensory pain component (Villemure and Bushnell 2002). Unpleasantness is moreover often increased in response to the thermal grill stimulation (Bouhassira et al. 2005; Lindstedt et al. 2011a). The subjective

evaluation of the intensity and unpleasantness of the thermal grill-induced sensations was done with a combined verbal-numerical rating scale (NRS; Gracely 2006; Lindstedt et al. 2011a) involving a continuous range from 0–100 and a set of verbal descriptors of the various scale increments. The 0 – < 20 range was used for the indication of no or non-painful thermal sensations [0 = *no sensation*; 10 = *warm*/*cold*; 20 = *grill pain threshold* (GPT)]. The ≥ 20–100 range was used for the assessment of the painful perceptions [20 = *grill pain threshold* (GPT); 30 = *very weak pain*/*unpleasantness*; 40 = *weak pain*/*unpleasantness*; 50 = *moderate pain*/*unpleasantness*; 60 = *slightly strong pain*/*unpleasantness*; 70 = *strong pain*/*unpleasantness*; 80 = *very strong pain*/*unpleasantness*; 90 = *nearly intolerable pain*/*unpleasantness*; 100 = *intolerable pain*/*unpleasantness*)]. It may be emphasized that the described subdivision implies that a pain rating of 20-NRS on our scale corresponds to a rating of 0-NRS (=*no pain*) on an ordinary scale, a 30-NRS rating is equivalent to 10-NRS (=*very weak pain*/*unpleasantness*), etc. The participants were explicitly instructed that the first part of the scale ranging from 0 to < 20-NRS-values should be used for the indication of non-existent or non-painful thermal sensations, whereas values ≥ 20 would always quantify intensity or unpleasantness levels related to the perception of pain. For the accurate assessment of their perceptions, the volunteers were allowed to use increments of 1.0 or 0.5 decimals on the NRS. They were furthermore instructed to rate the sensory-discriminative component of pain before the affective-motivational pain dimension. Pain ratings were orally delivered in intervals of 15 seconds during each thermal grill stimulation period (i.e. four sensory and four affective pain ratings per one-minute stimulation trial, three trials per condition; see Figure 2) since the dominant hand of the participants was positioned on the grill.

Self-report questionnaires

State- and trait anxiety. Inter-individual differences in state and trait anxiety were assessed with the Form Y of the State-Trait Anxiety Inventory (STAI; Spielberger et al. 1983). The questionnaire is based on 40 items and a 4-point Likert scale ranging from 1 = *not at all* to 4 = *very much so*. The first 20 expressions involve the state anxiety items, whereas trait anxiety is assessed with the statements numbered 21 – 40. Internal consistency (α = .95 and .93; Grös et al. 2007) and reliability of the STAI scales (Cronbach's α of.93; Balsamo et al. 2013) have been reported to be high.

Trait pain catastrophizing was assessed via the Pain Catastrophizing Scale (PCS; Sullivan et al. 1995). On the basis of a 5-point scale (0 = *not at all* to 4 = *all the time*), the

items of the rumination, magnification, and helplessness subscales of the PCS are related to feelings and thoughts associated with painful experiences of the past. Higher catastrophizing values (possible range 0–52) indicate greater emotional reactions to painful stimuli. The PCS has been classified as instrument with adequate to excellent internal consistency [coefficient alpha of total PCS: .87; rumination: .88; magnification: .66; helplessness: .78 (Sullivan et al. 1995)].

Dispositional Pessimism/Optimism. The revised version of the Life Orientation Test (LOT-R; Scheier et al. 1994) was used for the measurement of trait pessimism versus trait optimism in the participants (Herzberg et al. 2006). High scores indicate optimism and positive expectations for the future. The good validity and reliability of the LOT-R questionnaire have repeatedly been confirmed (Herzberg et al. 2006; Scheier et al. 1994).

The magnitude of *trait rumination* was determined with a short version of the Response Style Questionnaire (RSQ; Nolen-Hoeksema and Morrow 1991; Sütterlin et al. 2012). The 10 items refer to the subscales brooding (i.e. thoughtful contemplation of own problems and feelings of distress associated with negative mood and low or inexistent problem-solving behaviour) and reflection (i.e. inward-directed analysis of depressed feelings and potential engagement in adaptive actions) (Treynor et al. 2003). The self-report scores range from 0 = *never* to 3 = *always* and are summed as overall score reaching values between 0 and 30.

Interoceptive accuracy

IA was assessed with the heartbeat-tracking task (Herbert et al. 2012; Pollatos et al. 2007; Schandry 1981). Participants were asked to mentally count the number of heartbeats they felt during the time intervals of 25, 35, and 45 seconds. The experimenter orally informed the volunteers of the beginning and the end of the different time intervals. A pause of 60 seconds was implemented between all time periods. The participants were not allowed to use any additional help or strategies (e.g. measuring their pulse) and were not informed about the exact duration of the counting intervals to avoid heart beat estimations based on general knowledge. They were moreover instructed to sit comfortably during the task, to try to feel relaxed and to breathe regularly. An accommodation phase of 60 seconds preceded the actual cardiac perception measure to allow participants coming to rest and practicing the task. ECG-values were continuously recorded throughout the whole procedure.

The heartbeat perception score is considered as a valid index of IA. It bases on the comparison of the verbally reported with the ECG-recorded number of heartbeats and is calculated with the following formula: ⅓ ∑ [1 – (recorded heartbeats – reported heartbeats) / recorded heartbeats] (Herbert et al. 2012; Pollatos et al. 2007; Schandry 1981). The mean IA score is calculated across the three heartbeat-counting intervals and varies between 0 and 1. A higher score represents a smaller difference between reported and recorded heart rate i.e. higher IA. The test provides good test-retest reliability (about .81; Knoll and Hodapp 1992).

Suggestibility

The sensory suggestibility of the participants was assessed with the Warmth Suggestibility Scale developed by Gheorghiu et al. (2003). This standardized method bases on the application of various devices or procedures to simulate warmth stimuli or modifications of thermal sensations. In the present study, a flashlight, a medical examination lamp, a magnifying glass (diameter of 8 cm) and a contact thermode were used in the so-called initiation-, intensification-, and generalization suggestion tests to operationalize the assessment of the participants' suggestibility to the indirect sensory suggestions. The non-existence of the suggested flashlight- or lamp-induced warmth was controlled with a digital thermometer before starting the experiment. The volunteers were instructed to inform the experimenter as soon as they perceived the feigned warmth, respectively the amplification of the thermal sensation. To simulate warmth during the initiation test, it was suggested that the flashlight would approach the closed left eyelid of the participant during the stimulation period and that the light would be visible through the eyelid. In reality, the flashlight was held at a fixed distance of about 25 centimeters, thus precluding any perceivable heat stimulus. The intensification suggestion was operationalized with the lamp kept at about 50 centimeters over the dorsal side of the left hand of the volunteer and a magnifying glass moving from below the lamp towards the hand. It was implied that the lamp would release a noticeable stable heat and that the magnifying glass would focus the light of the lamp. By approaching the glass towards the hand of the participant, an intensification of the temperature of the focused warm stimulus would possibly be felt. The warmth generalization suggestion was based on an existing heat stimulus of 32°C (neutral temperature) delivered at the palm of the dominant hand via the heat contact thermode. It was indicated that due to symmetric or balancing physiological mechanisms, a similar sensation could emerge at the opposite side of the body, either in the right hand, arm, or in any other part of the right body side. The suggestibility tests were carried out in counterbalanced order. Participants 1–20 followed

the test order 1 (initiation), 2 (intensification), 3 (generalization), participants 21–40 the order 2, 1, 3, and participants 41–66 the order 3, 1, 2.

The three tests were applied once in each participant and always lasted 60 seconds. Each perception of the simulated warmth (initiation and generalization suggestion) resp. warmth modification (intensification suggestion) was verbally reported at the end of the respective trial and was scored one point. The absence of a sensory reaction was scored zero. The summed total score (range: 0–3) represented the individual and main suggestibility index. The time point at which the volunteer signalized that the simulated sensation was sensed or became more intense was considered as reaction time. This further measure of suggestibility was assessed with a stopwatch during each 0–60 seconds stimulation time range. For additional quantification of suggestibility, the evaluation of the distance observed between the magnifying glass and the hand at the moment where the intensification of the stimulation became real was assessed in centimeters. After all tests, the amount of confidence in the (non-) existence of the warmth sensations, respectively of concentration reached during the respective suggestion was rated. These additional indications on the personal extent of suggestibility were valued with a four-point Likert scale ranging from 1 = *not at all* to 4 v*ery good*. A smaller reaction time, a larger distance between the stimulus and the felt sensation, as well as a greater confidence and concentration level were considered as indicators of a higher suggestibility.

Experimental protocol

The different phases of the experimental protocol are depicted in Figure 3. The same experimenter conducted all the experimental sessions (each lasting about ninety minutes) in a temperature-controlled room (22° C). The participants delivered the previously completed trait questionnaires at their arrival in the lab and filled in their responses to the STAI state anxiety items. As soon as they were seated in the test chair, the main experimental phases were described and the stimulation equipment presented. The skin temperature at the participants' dominant hand was then measured with a digital thermometer. The experiment started with the assessment of the level of sensory suggestibility. A detailed explanation of the procedure was given before each trial. After the suggestibility assessment and detachment of the thermode from the hand of the participant, the thermal grill-related thermoelectric recirculating chillers and the contact heat stimulator were turned off to prevent all noise that might potentially hamper the subsequent heartbeat-tracking task. The ECG-electrodes were placed and a 10-minute baseline measure was done. Hereafter, IA was assessed with the heartbeat-tracking task

during three time intervals of 25, 35, and 45 seconds. In a next step, the thermal grill temperatures were set at 15°C and 41°C for the experimental thermal grill condition and the procedure started. On the basis of the combined verbal/numerical rating scale, the participants orally rated pain intensity and pain unpleasantness induced by the thermal grill tubes. Following the detachment of the ECG-electrodes, the volunteers assessed the magnitude of pain they had expected to experience during the experiment on a VAS, then they were debriefed and received their financial compensation.

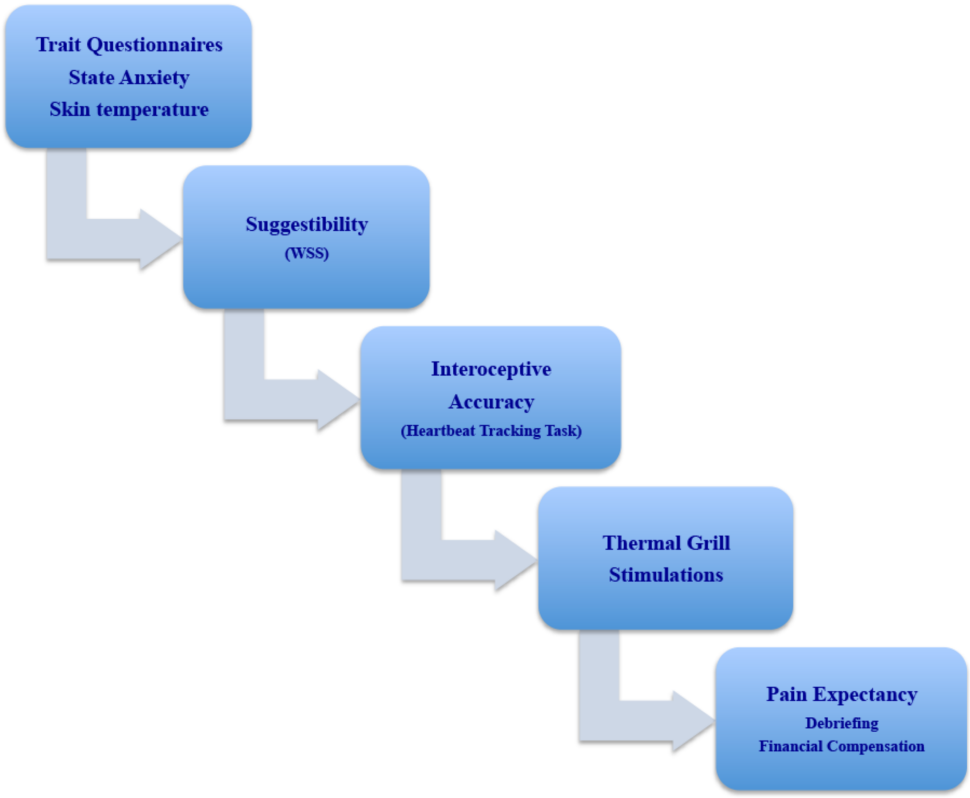

Figure 3: Experimental protocol

Statistical analyses

SPSS version 21 (IBM, Chicago/IL) was used for statistical analyses. The identification of responders and non-responders to paradoxical pain was based on mean pain intensity values. Mean scores were calculated by averaging the twelve reported pain values of each participant. Volunteers who had perceived more frequent and intense pain (Bouhassira et al. 2005) as expressed by higher mean scores were categorized as responders to the TGI. The responder/non-responder cut-off point in the present study was a ≥ 25-NRS score situated at equal distance between the 20-NRS score (GPT) and the 30-NRS score '*very weak pain*'. This score was chosen to allow the exclusion of highly variable near threshold ratings from the statistical analyses. It corresponds to 5/100-NRS on an ordinary 100 mm

NRS and is in the range of values considered as a reliable indicator of pain by Boettger et al. (2013). Subjects with no or low painful sensations (mean pain ratings < 25-NRS) were hence identified as non- or poor-responders. The same 25-NRS-criterion was used for the identification of the pain unpleasantness responders and non-responders. For both pain dimensions, the sample was split in a responder and a non-responder group in terms of pain intensity and of pain unpleasantness.

Descriptive statistics for all psychophysical, psychological, and psychophysiological measures were performed for the responder and non-responder groups (see Table 1). Normal distributions of the data were examined with Kolmogorov-Smirnov tests. The pain ratings and the different characteristics of both groups were compared and analyzed for differences using non-parametric tests for non-normally distributed pain-rating and suggestibility values and t-tests for independent samples in trait/state measures with normal distribution (see Table 1). Potential associations between the different variables were assessed with Spearman's resp. Pearson's correlations. All trait/state analyses were run with normalized trait/state data. *P*- and *t*-values < .05 (two-tailed) were considered significant.

Table 1: Absolute and statistical values of psychophysical, psychological and psychophysiological data[5]

Subjective pain ratings:	**RESPONDERS**		**NON-RESPONDERS**		**Non-parametric tests**
Pain Intensity:	***n* = 24** (44.4%)		***n* = 30** (55.6%)		**Independent samples**
	Mean, SD	**Min-Max**	**Mean, SD**	**Min-Max**	
	38.6 ± 9.8	25.4–63.3	14.4 ± 4.3	2.5–24.6	$^{a}p < .001^{**}$
Pain unpleasantness:	***n* = 19** (35.2%)		***n* = 35** (64.8%)		
	35.6 ± 11.1	25–64.2	11.6 ± 8.2	0–23.8	$p < .001^{**}$
Trait/State Questionnaires/Tests:					**Independ. samples *t*-tests**
	***n* = 27**		***n* = 27**		*t (df)* / *p*
Anxiety Trait	40.1 ± 8.7	26–60	39.8 ± 7.6	26–55	
Anxiety State	33.6 ± 9.7	0–47	30.8 ± 9.2	0–44	
Pain Catastrophizing	17.8 ± 9.5	2–31	16.1 ± 7.7	1–30	
Rumination	13.1 ± 4.9	3–25	10.9 ± 4.8	3–20	1.9 (49) / .05*
Optimism/Pessimism	16.1 ± 2.9	12–22	15.1 ± 4.3	6–23	
Interoceptive accuracy (IA)	.75 ± .23	.07– .99	.61 ± .25	.09– .95	2.0 (49) / .05*
Expectancy of pain	56.1 ± 20	0–85	59.6 ± 19.1	15–100	
Suggestibility (WSS):	***n* = 26**		***n* = 27**		
3 positive WSS tests:	5 participants		2 participants		
Positive Initiation test:	12 participants		10 participants		
RT (sec)	47.3 ± 17.3	5–60	51.9 ± 13.5	11–60	
Confidence	2.9 ± .8	1–4	3.2 ± .9	1–4	
Concentration	3.4 ± .7	2–4	3.4 ± .6	2–4	
Positive Intensification test:	19 participants		18 participants		
RT (sec)	29.5 ± 22.3	6–60	36.7 ± 20.6	3–60	
Distance (cm)	27.1 ± 15.4	5–45	24.1 ± 15.1	5–50	
Confidence	3.5 ± .5	2–4	3.3 ± .9	1.5–4	
Concentration	3.4 ± .8	1–4	3.7 ± .5	2–4	
Positive Generalisation test:	10 participants		13 participants		
RT (sec)	52.9 ± 11.4	16–60	50.4 ± 13.8	15–60	
Confidence	3.2 ± .8	2–4	3.1 ± .7	1–4	
Concentration	3.6 ± .6	2–4	3.6 ± .6	2–4	

[5] ^{a}p-values < .05 (two-tailed) were considered significant and values < .001 (two-tailed) as highly significant.

Logistic regression (LR) was performed to determine which of the psychological factors of interest significantly increased the likelihood of an occurrence of a painful and/or unpleasant thermal grill illusion and to control for the accuracy of our responder/non-responder classification. Pain intensity and pain unpleasantness were used as categorical (dichotomous) dependent variables. The mean scores of non-responders (< 25-NRS) were coded as 0 and higher pain values of responders (≥ 25-NRS) were coded as 1. All psychological and psychophysiological values were included in the LR as continuous independent variables resp. predictors, except for the suggestibility test scores (0 or 1), which figured as categorical predictors. The stepwise 'Forward Likelihood Ratio' procedure was employed to identify in groups of predictors those variables that provided the strongest predictive strength. Trait/state and suggestibility values were grouped separately and analyzed in distinct LR models. All predictors were logarithmically transformed (except for some categorical WSS predictors) and separately assessed for pain intensity and pain unpleasantness.

Results

Demographic and statistical characteristics

After exclusion of twelve participants of the total sample of tested volunteers, the data of a final sample of $N = 54$ participants [26 males, 28 females, M = 24.1 years (SD = 6.01), range 18–51 years] were analyzed. Mean pain intensity ratings were in line with other results described in the literature (Boettger et al. 2011, 2013; Bouhassira et al. 2005; see Table 1) and allowed classifying $n = 24$ participants into the category of responders (44.4%; 10 males, 14 females) and $n = 30$ into the category of the non-responders (55.6%; 16 males and 14 females) to the thermal grill illusion of pain (see Table 1). The categorization of pain unpleasantness ratings yielded $n = 19$ responders (35.2%; 10 males, 9 females) and $n = 35$ non-responders (64.8%; 16 males, 19 females) to unpleasantness of the grill stimuli (see Table 1). Overall, twenty-seven participants (50%) displayed paradoxical pain and/or pain unpleasantness responses. Sixteen responders (29.63%) reacted in both the sensory and the affective pain dimension. Twenty-seven volunteers did not ($n = 8$) or only poorly respond ($n = 19$). The assessment of the skin temperature of the participants' dominant hand revealed a mean value of 32.89°C and a SD of 3.11.

When comparing responder and non-responder values with respect to the sensory and the affective pain ratings, non-parametric tests disclosed a highly significant difference between groups in both pain dimensions ($p < .001$). Post hoc comparisons showed that responders and non-responders differed importantly in rumination and in IA levels ($p <$

.05). The investigation of the affective and cognitive personality trait and state data did mostly reveal higher mean scores in the responders than in the non-responders (see Table 1). The non-responders expected slightly more pain in the experiment than the responders and were somewhat more pessimistic. The analysis of the main WSS trials demonstrated that responders were more suggestible. Five responders felt the suggested warmth or increase of warmth in all three suggestibility tests, as compared to only two non-responding participants. During the generalization test of the WSS, the non-responders more often perceived the suggested warmth sensation in the contralateral body side. In general, the latter were slower in detecting the suggested heat sensation and perceived the simulated intensification stimulus at a smaller distance from the stimulation area. The suggestibility data of one participant were missing since this volunteer was familiar with the WSS. It should be stressed that the mentioned differences in pessimism, pain expectancy and suggestibility did not reach significance level (see Table 1).

Spearman's and Pearson's correlations

Pain intensity and pain unpleasantness highly correlated when all participants were included in the analyses ($r = .79$, $N = 54$, $p < .001$). In the same total sample, pain intensity and pain unpleasantness were significantly connected to rumination (intensity: $r = .28$, $N = 51$, $p < .05$; unpleasantness: $r = .36$, $N = 51$, $p < .01$). Correlations were also found between rumination and trait anxiety ($r = .52$, $N = 51$, $p < .001$), rumination and pain catastrophizing ($r = .44$, $N = 51$, $p \leq .001$) as well as between rumination and optimism/pessimism ($r = -.37$, $N = 50$, $p < .01$). IA correlated highly with trait anxiety ($r = -.40$, $N = 51$, $p < .005$), state anxiety ($r = -.30$, $N = 51$, $p < .05$) and optimism/pessimism ($r = .48$, $N = 49$, $p < .001$). Trait anxiety was most importantly associated to trait pain catastrophizing ($r = .46$, $N = 54$, $p < .001$), state anxiety ($r = .36$, $N = 54$, $p < .01$), and inversely to trait optimism/pessimism ($r = -.59$, $N = 52$, $p < .001$). In the group of the responding participants, optimism/pessimism was significantly related to IA ($r = .43$, $n = 23$, $p < .05$), and negatively to trait anxiety ($r = -.56$, $n = 25$, $p < .005$) and pain expectancy ($r = -.45$, $n = 25$, $p < .05$). Similar relationships as in the whole sample analyses were found in non-responders when considering correlations of rumination and IA with trait anxiety. The link between rumination-brooding values of the RSQ and those of the rumination dimension of the PCS reached significance in all groups (all $p < .05$).

Suggestibility was not linked to pain intensity sensations. The analyses of pain unpleasantness and suggestibility correlations in the whole sample however revealed a strong correlation with concentration in the intensification suggestion ($r = -.28$, $N = 53$, $p <$

.05) and with reaction time in the generalization suggestion (r = .35, N = 53, p < .05). In non-responders, an important negative association between the affective pain component and reaction time in the intensification suggestion (r = −.40, N = 27, p < .05) could be observed.

Logistic regressions (LR)

Predictors of the thermal grill illusion of pain

Trait/state variables. In the context of pain intensity, we focused in our first LR model on the potential impact of trait pessimism/optimism, trait pain catastrophizing, trait anxiety, trait rumination, pain expectancy, and IA on the likelihood that participants experienced the TGI. The statistically significant full model [X^2 (2, N = 40) = 15.14, p < .005] showed that rumination and IA significantly contributed to the predictive ability of the model (all p < .05). The other independent variables did not add to the probability of a TGI occurrence. The model including rumination and IA explained between 31% (Cox and Snell R square) and 42% (Nagelkerke R square) of the variance in the TGI perception. 77.5% of the cases were correctly classified (i.e. 76.5% of the responders and 78.5% of the non-responders to the TGI). Rumination was the strongest predictor of paradoxical pain and presented an odds ratio of 35.86 (CI 2.33, 551.67; see Table 2). This result specifies that in case the rumination characteristic is under control in the model, ruminative persons are about 35 times more likely to perceive the illusion of pain than those who ruminate less. The odds ratio for IA was 20.19 (CI 1.80, 226.81; see Table 2), which signalizes that individuals who perceived their heartbeats more accurately had a 20 times higher probability to feel the paradoxical pain than less interoceptively accurate candidates. The second LR model we used included the suggestibility variables. No potential predictor of the TGI could be identified in this model.

Trait/state – interaction terms. The study of interacting trait/state predictors of the TGI outcome showed that rumination also considerably supported the paradoxical pain elicitation when interacting with state anxiety [X^2 (1, N = 49) = 7.73, p < .05; .15 (Cox and Snell), .20 (Nagelkerke)], pain expectancy [X^2 (1, N = 50) = 6.86, p < .05; .13 (Cox and Snell), .17 (Nagelkerke)], optimism/pessimism [X^2 (2, N = 51) = 12.85, p < .005; .22 (Cox and Snell), .30 (Nagelkerke)], and IA [X^2 (1, N = 48) = 10.93, p < .01; .20 (Cox and Snell), .27 (Nagelkerke)] (see Table 2). Between 63.3 and 75% of participants were correctly classified in these interaction models. Even a three-factor interaction term involving rumination, IA, and pain expectancy contributed significantly to the TGI prediction (p < .05). The predictive ability of this model was important [X^2 (1, N = 48) = 8.84, p < .05] and

explained between 17% and 22% of the variation in the pain intensity outcome. 75% of the participants (71.4 responders and 77.8% of non-responders) were correctly classified in the model. It could be seen that overall the likelihood of the appearance of the TGI was one to two times higher in those individuals with interacting personality features than in those without related characteristics (odds ratios varied between 1.11 and 2.81; see Table 2). It was further observed that trait anxiety and trait pain catastrophizing did not act on the probability of the TGI appearance. State anxiety, optimism/pessimism, and pain expectancy only had an effect on the prediction of pain when associated with perseverative thinking.

Table 2: Significant predictors of pain intensity and pain unpleasantness perceptions during thermal grill stimulation[6]

	B	S.E.	Wald	df	[a]*p*	Odds Ratio	95.0% C.I. for Odds Ratio	
Predictors for pain intensity:							Lower	Upper
Rumination	3.58	1.39	6.59	1	.01*	35.86	2.33	551.67
Interoceptive Accuracy (IA)	3.01	1.23	5.93	1	.01*	20.19	1.80	226.81
Interaction Terms:								
State Anxiety x Rumination	.51	.21	5.75	1	.02*	1.67	1.10	2.55
Pain Expectancy x Rumination	.46	.20	5.40	1	.03*	1.48	1.04	2.13
Pessimism/Optimism x Rumination	1.03	.36	8.13	1	.004**	2.81	1.38	5.70
IA x Rumination	.53	.20	7.38	1	.007*	1.71	1.16	2.51
IA x Pain Expectancy x Rumination	.10	.04	6.49	1	.01*	1.11	1.02	1.20
Predictors for pain unpleasantness:								
Rumination	3.42	1.62	4.45	1	.03*	30.72	1.28	738.85
Suggestibility (WSS):								
Intensification Test – Concentration	-.88	.45	3.71	1	.05*	.42	.17	1.01

Predictors of pain unpleasantness perceptions

Trait/state variables. Regarding the prediction of pain unpleasantness outcomes in the present research, the inclusion of all previously described trait/state predictors in the logistic regression analyses again identified rumination as major influencing factor in the significant full model [X^2 (1, N = 40) = 6.68, p < .05]. The predictor clarified between 15% (Cox and Snell) and 23% (Nagelkerke) of the dispersion in pain unpleasantness. The model

[6] [a]*p*-values < .05 (two-tailed) were considered significant and values < .005 (two-tailed) as highly significant.

allowed categorizing 75% of the volunteers in the appropriate group (i.e. 96.7% responders, 10% non-responders). Ruminators were 30 times more likely (Odds ratio of 30.72; CI 1.28, 738.85) to distinguish the repulsiveness of the thermal grill than non-ruminating individuals. Interacting trait/state variables did not have a predictive probability effect on the affective-motivational pain component.

Suggestibility-related LR results demonstrated that concentration assessed during the intensification suggestion significantly predicted the likelihood of pain unpleasantness perceptions induced by the grill ($p \leq .05$). The model performed significantly well [X^2 (1, N = 53) = 4.15, $p < .05$] and explained 7% to 10% of the variance in the dependent variable. Overall, 69.8% of the volunteers were correctly classified. The odds ratio of .42 inferior to 1 specified that less concentrated participants were more likely to report unpleasantness (see Table 2).

Discussion

The psychophysical results of the present research are in agreement with previously described thermal grill-related pain ratings (Boettger et al. 2011, 2013; Bouhassira et al. 2005) and demonstrate that the applied temperature combination of 15°C and 41°C (26°C difference) yielded similar intensity and unpleasantness ratings of paradoxical pain. The evaluation of the pain scores and personality variables allowed classifying and characterizing responders and non-responders to the thermal grill stimulation paradigm. In this context, it should be emphasized that there is no generally accepted criterion for the discrimination of the two categories. As mentioned in the methods section, we chose a cut-off point of ≥ 25-NRS situated at equal distance between the 20-NRS score (GPT) and the 30-NRS score 'very weak pain'. This value allowed us to exclude highly variable near threshold ratings from the statistical analyses. It corresponds to 5/100-NRS on standard 100 mm rating scales and hence is in the range of values considered as reliable indicators of pain (Boettger et al. 2013).

With regard to the inter-individual differences in TGI sensitivity, our results are to the best of our knowledge the first providing evidence that psychological factors in the form of cognitive and affective personality characteristics have an impact on the probability of the TGI occurrence. It could especially be established that individuals displaying high levels of trait rumination and interoceptive accuracy are more prone to perceive the illusive pain in response to the innocuous TG-stimulation. In addition, these novel findings may be relevant in the context of central neuropathic pain, which has been shown to share common neural mechanisms with respect to dysfunctional interactions between thermo-sensory and

nociceptive processing (Craig et al. 1996, Craig 2008, Kern et al. 2008). The identification of significantly involved psychological factors may therefore be seen as an important contribution to the elucidation of central neuropathic pain processing and may in the longer term be relevant for the development of novel assessment and treatment strategies.

Rumination and the thermal grill pain illusion

The strong role of rumination in the prediction of the pain illusion indicates that individuals characterized by perseverative and negative reflecting on their feelings or problems and by inactive problem-solving behaviour (Nolen-Hoeksema et al. 2008) are more pain sensitive in response to non-noxious stimulation and can feel pain where no pain should be felt. It may further be assumed that maladaptive coping with adverse contexts (Geers et al. 2008), negative expectancies of present and future outcomes (Goodin et al. 2013), and failures in deflecting attention from anticipated or on-going painful stimulations (Arntz et al. 1994; Crombez et al. 1998; Peters et al. 2000; Van Damme et al. 2004) make ruminators feel more distressed and anxious (Tang and Gibson 2005; Smith and Alloy 2009) and thus more susceptible to the TGI. In pain studies with suprathreshold noxious stimuli, it was suggested that the cognitive rumination feature may primarily influence pain perceptions when considered as a sub-factor of pain catastrophizing (Sullivan et al. 1995). In the present pain context however, the rumination trait did not act in combination with pain catastrophizing since its assessment on the basis of the Pain Catastrophizing Scale (PCS-R) did not reveal a meaningful impact. Instead, we uncovered the significant predictive capacity of the stand-alone rumination characteristic when assessing it with a pain-unspecific questionnaire, i.e. the RSQ (Nolen-Hoeksema and Morrow 1991). Nevertheless, both rumination measures, as well as rumination and pain catastrophizing correlated with each other.

Interoceptive accuracy and the thermal grill pain illusion

The relationship between high interoceptive accuracy and enhanced affectivity or increased pain perceptions established in classical pain research (Pollatos et al. 2007, 2012) could interestingly also be observed in the present thermal grill investigation. It could be demonstrated that the ability to perceive bodily signals accurately increases the likelihood of the illusion of pain experience, a finding that may also be relevant in the context of neuropathic pain where dysfunctional thermo-sensory processes are commonly observed. The effect may possibly be explained by the circumstance that the cognitive processing of bodily cues is subjected to an emotional evaluation. With regard to the more intense emotions displayed by interoceptively accurate individuals (e.g. anxiety; Critchley et al.

2004; Krautwurst et al. 2014; Pollatos et al. 2007; Wiens et al. 2000), it has been stipulated that these strong feelings may interfere with the described affective appraisal so as to render the latter dysfunctional to a variable extent (Fairclough and Goodwin 2007; Garfinkel and Critchley 2013; Sütterlin et al. 2013; Wölk et al. 2013). In this sense, greater accuracy of estimate in the heartbeat-tracking task often revealed an association between negative cognitive appreciation of somatic cues and increased interoceptive sensitivity (Ehlers and Breuer 1996; Wölk et al. 2013). Similar impaired affective assessment of somatic signals was observed in patients displaying poorer cognitive-affective processing during decision-making processes and in healthy participants when analyzed in health anxiety and symptom report contexts (Krautwurst et al. 2014). Considering that misjudgments of interoceptive cues are held responsible for the reported enhanced somatic symptom experiences (Critchley et al. 2004) or over-reports of physical symptoms (Barsky and Borus 1999), it was proposed that anxiety-induced increases in interoceptive processing may not only maintain anxiety, but also pain which is considered to be an indicator of the physiological condition of the body (Craig 2002; Wiech and Tracey 2009). All these findings convincingly support the current finding that more accurate heartbeat perceivers are more probable to display intense paradoxical pain sensations.

Interacting personality traits and the thermal grill pain illusion

Beside the influence of rumination per se, it could be shown here that the same cognitive characteristic also significantly increased the prediction of the TGI when interacting with anxiety, pain expectancy, pessimism, and IA. A relationship between rumination and anxiety or hostile expectations has already been demonstrated in scientific literature on depressive disorders (Nolen-Hoeksema 2000, Nolen-Hoeksema et al. 2008; Smith and Alloy 2009). Repetitive thoughts have been claimed not only to predict chronicity of depressive disorders, but also anxiety symptoms (Nolen-Hoeksema 2000), their amplification and maintenance (Segerstrom et al. 2000). Other research findings corroborated the link between rumination and anxiety by disclosing a mediating effect of rumination on the relationship between neuroticism and anxiety, respectively depression (Muris et al. 2005). The content of primarily negative ruminative thoughts, as well as pessimistic orientations and adverse expectations on present or upcoming events often seem to accompany persistent thinking (Smith and Alloy 2009). In pain research, anxiety, pain expectancy, and pessimism have mainly been related to pain catastrophizing and not to perseverative thinking since rumination is considered as a sub-factor of the multidimensional pain catastrophizing construct (Crombez et al. 1998; Edwards et al. 2006; Sullivan et al. 2001a, 2005). It has thus been recognized that increased anxiety (Sullivan et

al. 2001b) and dispositional pessimism (Sinclair 2001) trigger hyperalgesia when these variables are concomitant to high pain catastrophizing. Other investigations on the impact of catastrophizing on pain perceptions and emotional distress in turn revealed that expectancy of pain mediated the relationship between catastrophizing and pain sensitivity in healthy participants (Sullivan et al. 2001b). It could furthermore be established that the magnitude of pain intensity and pain unpleasantness ratings depends on the intensity of pain an individual expects during noxious stimulation (Atlas and Wagner 2012; Tracey 2010). The more pain somebody anticipates, the more pain he will feel (Arntz et al. 1994). This relationship also reinforces expectation-based nocebo and placebo responses when influenced by anxiety and worry (Benedetti et al. 2007; Sawamoto et al. 2000). In classical pain research the interaction of rumination and IA has so far not been explored. Our findings may suggest that rumination-related negative cognitions of responders and the extent of IA, as a measure for the sensitivity to somatic signals and an indicator of emotional processing intensity, may partly interdepend. Perseverating negative thoughts and concomitant intense emotions may wind each other up and by this way exacerbate paradoxical pain sensitivity. The potentially facilitating effect of pain expectancy in the three-factor interaction with rumination and IA observed in the present study further supports the accuracy of a TGI prediction in individuals displaying negative evaluations of bodily signals. Taken together, our interaction results seem to imply that the induction of thermal grill-related pain sensations depend on affective characteristics like state anxiety, pain expectancies, dispositional pessimism, or interoceptive precision whilst cognitive factors like perseverative thoughts were possibly mainly involved in the maintenance of accompanying emotions, cognitions, and consequently paradoxical pain.

Suggestibility and rumination in thermal grill-induced pain unpleasantness

The present research revealed that an individual's level of suggestibility interestingly played a role in the probability of the occurrence of the affective component (unpleasantness) of the TGI rather than of the sensory-discriminative component (paradoxical pain intensity). This finding implies that more suggestible persons express preferentially unpleasantness-related sensations. It might be interesting to analyze the same suggestibility-pain unpleasantness relationship in neuropathic pain patients. In case of positive affirmation of the unraveled effect, this result might contribute to the understanding of pathological pain states that are independent of noxious input. The in literature described direct relationship between suggestibility and pain-related placebo- or nocebo effects (De Pascalis et al. 2002; Staats et al. 1998) should also be kept in mind in the clinical context.

It could moreover be observed that the cognitive factor rumination had a very strong predictive impact on the affective-motivational pain component related to the thermal grill stimulation. Other personality features did neither act alone nor in interaction with others on affective aspects of pain. The suggestibility and rumination results seem to point towards differential effects of psychological characteristics on thermal grill-related pain unpleasantness and intensity. Considering the scarcity of findings on the impact of suggestibility or rumination on pain unpleasantness in classical pain conditions, it may be hypothesized that negative cognitive processing in combination with enhanced suggestibility fostered adverse pain expectancies and were thus accountable for the unpleasant pain sensations in the current research. Further systematic research will be needed to elucidate these assumed relationships.

Conclusion

We were able to confirm our hypothesis that the psychological factors rumination, interoceptive accuracy, and suggestibility are substantially involved in the individual pre-disposition to reporting painful sensations in the thermal grill paradigm. Further studies aiming at characterizing the impact of additional potentially involved psychological constructs (like emotional self-regulation) will be conducted to further the understanding of thermal grill-related illusive pain and concomitantly the elucidation of dysfunctional thermo-sensory processing as observed under conditions of neuropathic pain. In the long term, the respective sets of data may contribute to the development of novel assessment and treatment strategies.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RS, SS, and FA elaborated the study design. RS carried out the experiment, performed the statistical analyses, and drafted the manuscript. SS interpreted the data and critically revised the drafted manuscript. FA provided supervision at each stage of the study. All authors read and approved the final manuscript.

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3.3 Study 3: The perception of the thermal grill illusion of pain is affected by the magnitude of heart rate variability at rest

Abstract

Self-regulation mechanisms play a crucial role in the modulation of pain and are governed by prefrontal inhibitory processes. Vagally mediated heart rate variability (HRV) is a marker of neural inhibitory capacity and is related to top-down processes by regulating associated thoughts, emotions, behaviour, and physiological activation. The present study aimed at investigating whether resting HRV as a physiological correlate of trait self-regulatory capacity explains inter-individual differences in the perception of the thermal grill illusion of pain (TGI). Fifty-two healthy participants were stimulated with a temperature combination of 15°C and 41°C set at a water-bath driven thermal grill device. Sensory and affective pain perceptions were evaluated with numerical rating scales. The results showed that participants with higher resting vagal activation were more likely to perceive the pain illusion ($p < .05$) than subjects not displaying similar levels of self-regulation strength or HRV. Especially time-domain components of HRV and normalized respiratory sinus arrhythmia values predicted the probability of a TGI occurrence ($p < .05$). The present results support previous findings indicating an impact of personality traits on the individual disposition to paradoxical pain perceptions. Since the thermal grill is considered as a fundamental tool in the investigation of central neuropathic pain processes, the outcome of the current research may also suggest an influence of the self-regulation ability characteristic on mechanisms underlying these pain syndromes.

Key words: heart rate variability, paradoxical pain, responder, thermal grill illusion, emotional self-regulation.

The perception of the thermal grill illusion of pain is affected by the magnitude of heart rate variability at rest

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1. Introduction

Pronounced unpleasantness and negative affect accompany the sensory experience of pain. Both components of pain may be intensified by adverse cognitive and emotional processes, as e.g. expressed by increased attention to pain, expectation of pain, anxiety, or pain catastrophizing (Arntz et al., 1994; Sullivan et al., 2001; Van Damme et al., 2002). Increased blood pressure (BP) and heart rate (HR) often reflect acute pain and associated thoughts or emotions (Loggia et al., 2011). Alterations in baroreceptor reactivity and concomitant changes in cardiac rhythm and BP related to these processes contribute to the modulation of pain sensitivity (Bruehl and Chang, 2004; Edwards et al., 2003; Guasti et al., 2002; Randich and Maixner, 1984; Thayer et al., 2012). Self-regulation ability has been shown to support the flexible control of negative emotional influences and cognitive responses to emotional stimuli during challenging demands (Park & Thayer, 2014; Segerstrom and Solberg Nes, 2007; Solberg Nes et al., 2009; Thayer and Lane, 2000; Thayer et al., 2009, 2012). Since pain has been conceptualized as a homeostatic emotion (Craig, 2003), it has been claimed that the regulating actions are also promoted during pain states warranting adaptive behavior in the face of noxious challenges. A flexible and effortful coping is assured and the organism's homeostatic drive for an equilibrated body condition is satisfied (Appelhans and Luecken, 2008; Craig, 2003). Chronic pain conditions have in contrast been related to reduced executive functioning and deficits in self-regulation (Solberg Nes et al., 2009).

The neural substrates of all homeostatic regulation processes consistently overlap in the prefrontal cortex (PFC; Thayer et al., 2009, 2012). Especially the medial prefrontal cortex (mPFC) plays an important role in the cerebral processing of cerebral depictions of inner and outer circumstances ensuring flexible behavioral and autonomic nervous adaptability. This common higher order regulation system coordinates actions by means of inhibitory processes. The mPFC pathways are linked to the central autonomous network (CAN), a neural system responsible for visceromotor, neuroendocrine, and behavioural homeostatic processes (Benarroch, 1993; Thayer and Lane, 2000) and are connected to subcortical structures like the amygdala, anterior cingulate cortex, insula, hypothalamus and brainstem nuclei (Thayer et al., 2009). The CAN is considered as a key feature in reciprocal cortico-cardiac interactions in charge of a flexible adaptation of the organism to situational demands. Thayer and Lane (2000) included the CAN in the neurovisceral integration model and suggested that it constitutes a functional unit

regulating psychological and physiological control processes via the described neural circuitry and related inhibitory processes.

In recent years, vagally mediated heart rate variability (HRV) during rest has been used as an index of prefrontal inhibitory functioning, of cognitive control of responses to emotional stimuli (Park and Thayer, 2014), and in a more general sense of the individual self-regulation ability predisposition (Segerstrom and Solberg Nes, 2007). Vagally mediated HRV at rest is a proxy for tonic vagal activation and can predict emotional self-regulation capacity in healthy and clinical samples (Appelhans and Luecken, 2006, 2008; Koval et al., 2013; Park et al., 2014; Solberg Nes et al., 2009; Thayer et al., 2009, 2012). Higher resting HRV has been associated with more adaptive and flexible homeostatic responses, positive emotionality, good health, and psychological recovery. Interestingly, both resting HRV and self-regulation features are considered as individually varying but partially inheritable, stable trait characteristics (Sinnreich et al., 1998; Thayer et al., 2009; Wang et al., 2005). In classical pain models, a relationship between pre-experimentally measured resting HRV values and pain sensitivity in response to subsequent noxious stimulation has been established (Appelhans and Luecken, 2008). Phasic HRV assessed during acute experimental pain recurrently revealed that lower vagal reactivity was linked to higher pain sensitivity and emotionality (Koenig et al., 2014; Pollatos et al., 2012).

The thermal grill paradigm consists in applying interlaced non-noxious warm and cold temperatures to the skin and has commonly been used for the induction of paradoxical pain sensations, also called thermal grill illusion of pain (TGI; Craig and Bushnell, 1994; Thunberg, 1896). In addition, this method has been claimed to be a valid model for the study of central neurophysiological processes involved in neuropathic pain conditions (Craig and Bushnell, 1994; Craig, 2008; Kern et al., 2008) and of the underlying impact of psychological factors like sad mood, depression, and schizophrenia (Boettger et al., 2011, 2013; Piñerua-Shuhaibar et al., 2011). However, several studies have shown that only about one third to half of the tested individuals express the respective TGI in response to this procedure (Boettger et al., 2011, 2013; Bouhassira et al., 2005, Lindstedt et al., 2011). In this context, our laboratory has shown that the psychological traits rumination and interoceptive accuracy are major predictors of the occurrence of the TGI (Scheuren et al., 2014).

In the present study, we hypothesized that differences in the extent of dispositional self-regulation capacity may be an additional source of inter-individual variance in the susceptibility to pain responses related to non-noxious stimulation. We tested this

hypothesis by investigating relationships between psychophysical responses to thermal grill stimulation and different parameters of HRV during rest.

2. Methods

The present investigation is part of a more extended study and refers to a common sample of participants (see Scheuren et al., 2014). Several paragraphs of the following methods section (2.1., 2.2., and 3.1.) reflect procedural descriptions that are described in more detail in the cited article.

2.1. Participants

Sixty-six healthy students and staff members of the University of Luxembourg were recruited. The study was approved by the National Research Ethics Committee and was conform to the ethical guidelines of the International Association for the Study of Pain (IASP; Charlton, 1995). Exclusion criteria were previous or current psychological- (e.g. depression, anxiety disorder), cardiovascular-, neurological-, pain-, and skin-related problems, as well as drug and pain medication intake 24 hours before the experimental session. All health-related items were addressed with a medical history questionnaire. One volunteer could not participate because of depressive symptoms. The incomplete data of another volunteer (i.e. technical problems with the thermal grill) were not analyzed. Ten participants rated pain sensations in response to control conditions and were excluded from the study. Two participants could not be included in the sample because of equipment failure-related incomplete HRV data. The final total sample involved 52 participants (28 females). The mean age in the sample was 24.1 years (SD = 6.08, range: 18–51 years). All volunteers signed the informed consent and received financial compensation.

2.2. Material and Measures

2.2.1. Thermal grill device

A custom-built and water-bath driven thermal grill device (Curio, I., PhD, Medical Electronics, Bonn/Germany) composed of eight alternating cold and warm glass tubes (rectangular surface of 20 x 10 cm; contact area of the skin to the glass tubes of about 71

cm²) was used to elicit the TGI. Two separate thermoelectric recirculating chillers (T255P, ThermoTek, Inc.) regulated the temperatures of the water delivered to the grill tubes. A digital thermometer (PL-120 T2, Voltcraft; visual display of T1-T2 temperatures in °C) allowed a continuous control of the correct temperatures.

During the experimental thermal grill condition, participants were stimulated with a fixed temperature combination of 15°C and 41°C (Boettger et al., 2011; Bouhassira et al., 2005; Lindstedt et al., 2011) applied to the palmar side of the dominant hand. A cuff inflated with a sphygmomanometer was used to induce a weak pressure of 0.7 MPa (0.071 kp/cm²) holding the hand at the grill surface. Thermal stimulation trials lasted one minute and were repeated two times. In between, the hand was removed from the grill tubes and an inter-stimulus-interval (ISI) of three minutes was observed. The experimental condition was followed by two control conditions, a first one with a temperature combination of 15°C and 32°C (mean skin temperature) and the second one with a combination of 41°C with 32°C. The same temporal procedure was used in all conditions.

2.2.2. Physiological assessments

During 10 minutes, a standard precordial lead II electrocardiogram (ECG100C; 0.5 Hz high pass filtering, R-wave output mode, signal gain 500) was performed in the comfortably sitting (reclined test chair, ±110°) and relaxed participants. Disposable pre-gelled Ag-AgCl electrodes (diameter 35 mm, EL502) were placed below the right clavicle and below the left lower rib. A similar Ag-AgCl electrode positioned below the right lower rib served for grounding. Heart rate (HR) data were continuously recorded with an MP150 Data Acquisition System and monitored by means of the AcqKnowledge Software package (BIOPAC Systems Inc., USA).

2.2.3. Psychophysical measures

Participants evaluated pain intensity and pain unpleasantness sensations perceived during thermal grill stimulation by means of 100 mm numerical rating scales (NRS; Gracely, 2006; Lindstedt et al., 2011). They were instructed to refer to a list with verbal descriptors of the various numerical scale increments: 0 = *no sensation*; 10 = *warm/cold*; 20 = *grill pain threshold* (GPT); 30 = *very weak pain/unpleasantness*; 40 = *weak pain/unpleasantness*; 50 = *moderate pain/unpleasantness*; 60 = *slightly strong pain/unpleasantness*; 70 = *strong pain/unpleasantness*; 80 = *very strong*

pain/unpleasantness; 90 = *nearly intolerable pain/unpleasantness*; 100 = *intolerable pain/unpleasantness*. In our thorough instructions we emphasized that values ranging from 0 to 20-NRS should be used to rate no- or non-painful warm or cold sensations while values ≥ 20-NRS should quantify the intensity and unpleasantness of pain sensations. The magnitude of the sensory-discriminative component of pain was measured before the affective-motivational pain dimension. During each one-minute thermal grill stimulation the instructor orally invited the participants to rate the perceived perceptions in intervals of 15 seconds.

2.3 Experimental Protocol

At the beginning of the laboratory session, an overview of the experimental procedures was given to the seated participants. After familiarization with the pain rating scales, the ECG-related electrodes were placed (see section 2.2.2.). A pre-experimental 10-minute ECG recording was performed in resting condition. Subsequently, the temperature combination of 15°C and 41°C was set at the thermal grill and the experimental grill stimulation phase was initiated (see section 2.2.1.). The subsequent control conditions were preceded by a time interval of about 10 minutes to allow the water-bath driven grill temperatures to adjust. At the end of the grill stimulation protocol, the ECG-electrodes were detached and the participants were debriefed and financially compensated. All experimental sessions were run in a temperature-controlled room (22° C) by the same investigator.

2.4. Reduction of ECG-related data

Artifact identification, correction, and HRV analysis were performed via ARTiiFACT software (V. 2.07; Kaufmann et al., 2011). R-R intervals (RRI) were extracted from the ECG measurements recorded during the pre-experimental resting condition (last five minutes of the 10-min recordings). We included time- and frequency domain measures as well as normalized respiratory sinus arrhythmia (RSAnorm) values in our analysis since these parameters have been considered as equally valid indicators of vagally mediated HRV (Task Force of the European Society of Cardiology, 1996; Kaufmann et al., 2012), which in turn constitutes a marker for cognitive and emotional self-regulation ability (Park et al., 2014; Segerstrom and Solberg Nes, 2007; Thayer et al., 2009). Both time- and frequency domain measures of HRV have been shown to provide high temporal stability, reliability, and reproducibility (Bertsch et al., 2012; Task Force, 1996).

Evidence has also been given for the repeatability (Ritz et al., 2001; Stein et al., 1995) and stability of the RSAnorm index (Sinnreich et al., 1998).

Time domain measures. Mean heart rate, RMSSD (square root of the mean squared differences of successive NN intervals) and pNN50 (the proportion derived by dividing NN50 by the total number of NN intervals; the NN intervals correspond to elapsed time between subsequent ECG-R-peaks in milliseconds) are reported in the current study as time domain measures (Task Force, 1996).

Spectral frequency measures involved high-frequency (HF, 0.15–0.4 Hz) values as expressed in power (ms^2) and normalized units (n.u.).

Respiratory sinus arrhythmia. RSA is a cardiorespiratory phenomenon resulting from the interaction between cardiovascular and respiratory systems and reflecting cardiac vagal tone (Grossman and Taylor, 2007; Task Force, 1996). In the current study, the normalized RSA index (also called Hayano index; Hayano et al., 1990) was used as an indicator of vagal activity and inhibitory capacity. It has been suggested that the normalization of HF (ms^2) with mean interbeat interval allows correcting for the potential influence of sympathetically induced changes in mean RRI (Grossman and Taylor, 2007; Hayano et al., 1990; Kaufmann et al., 2012).

2.5. Statistical analyses

All data were statistically analyzed with SPSS, version 21 (IBM, Chicago/IL). An equipment failure caused incomplete HRV values in two participants. Their data were list-wise excluded. The HF (ms^2) data of six volunteers were pairwise excluded because of outlying values.

With regard to the division of the sample into groups of participants perceiving pain in response to thermal grill stimulation (responders) and non-responders, mean pain intensity values were calculated by averaging the twelve NRS-ratings obtained during thermal grill stimulation phases. In the absence of a common definition of responders and non-responders, we chose a 25/100-NRS cut-off point for the classification of the participants into the respective groups. Mean pain values ≥ 25-NRS corresponded to 5/100-NRS on an ordinary (not displaying a 0–20-NRS pre-pain range) and were in line with the rating value of ≥ 6/100-NRS that Boettger et al. (2013) had used as an indication of pain and as a responder/non-responder classification index in their thermal grill study. Our cut-off point also reflected “more frequent and intense” paradoxical pain perceptions Bouhassira and co-authors (2005) had used as criterion for the distinction between responders and non-responders. The cut-off value was moreover situated between scores

of 20-NRS (GPT) and 30-NRS (very weak pain) to rule out contaminating variability in the near threshold range. The same 25-NRS-based procedure was used for the identification of responders and non-responders to the affective-motivational component of paradoxical pain as measured by the level of unpleasantness.

Mean pain intensity and pain unpleasantness ratings, HR, and HRV parameters were analyzed for the final total sample and separately for the groups of responders and non-responders. Normality of distribution was verified with the Kolmogorov-Smirnov test (Lilliefors significance correction). The data were log-transformed when the assumption of normality was violated. Post-hoc comparisons tested potential differences between responder and non-responder values. Pearson's correlation analyses were performed to identify possible associations between vagal activation components and pain ratings.

The data of the final total sample was included in logistic regression (LR) analyses to examine whether vagal activation indices predicted the probability of the occurrence of the sensory respectively of the affective component of the TGI. Separate analyses were run for pain intensity and pain unpleasantness. Thermal grill responder values were coded as 1, non-responder data as 0. HRV parameter [i.e. RMSSD, pNN50, HF (ms^2) and RSAnorm] values were analysed as absolute and logarithmically transformed values and figured as continuous independent variables in the LR analyses. The pain rating data were used as categorical (dichotomous) dependent variables.

3. Results

3. 1. Thermal grill stimulation responders and non-responders

Mean pain rating values are presented in Table 1 with respect to the whole sample and separately for pain intensity respectively pain unpleasantness responders and non-responders. The differences in pain intensity and pain unpleasantness perceptions between the groups of responders and non-responders were highly significant (see Table 1).

Less than half of the sample only perceived the sensory-discriminative component of thermal grill-related pain (n = 23), whereas n = 29 did not display any intensity ratings in response to the stimulation paradigm. Seventeen participants only responded with pain unpleasantness as compared to thirty-five pain unpleasantness non-responders. Participants displaying intensity *and* unpleasantness ratings following thermal grill stimulation or either one of these two response categories were included in the global group of responders (n = 25). According to this classification, the group of non-responders comprised 27 participants (see Figure 1).

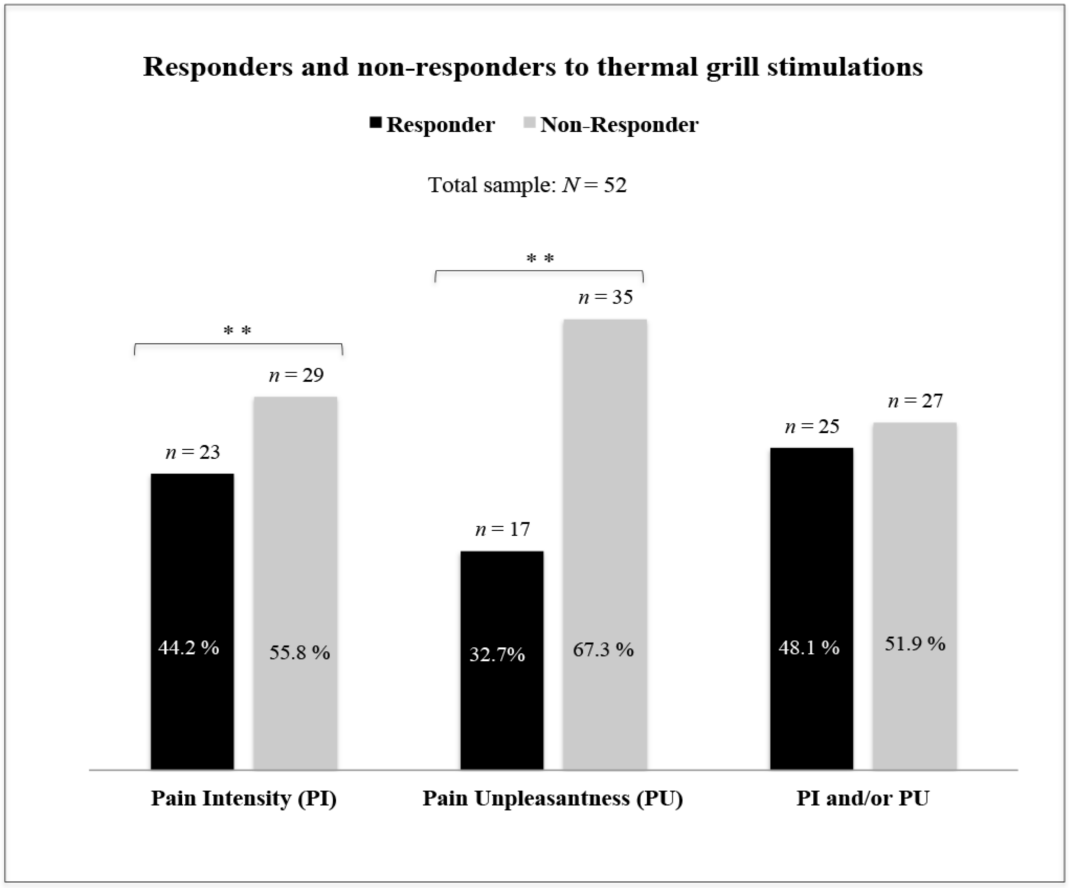

Figure 1: Proportions of responders and non-responders to the thermal grill stimulations with respect to pain intensity, pain unpleasantness, and the combination of both parameters.

3.2. Cardiac activity

3.2.1. Descriptive statistics and post hoc comparisons

Mean resting HR and HRV values of the final total sample respectively of responders and non-responders are presented in Table 2. HR measured at rest was slightly lower in paradoxical pain intensity responders as compared to non-responders, without however reaching significance. We observed a tendency of a correlation between mean HR and pain unpleasantness (r = .43, n = 17, p = .08) in the responder group.

Concerning vagally mediated HRV at rest, a physiological indicator of trait self-regulation ability, pain intensity responders showed greater vagal activation than pain intensity non-responders (see Table 2). In particular, RSAnorm [t (27) = –2.53, $p < .05$, r = .44; see Table 2], pNN50, and HF (ms^2) measures were higher in pain intensity responders as compared to non-responders to the thermal grill stimuli. We also observed a difference in RSAnorm [F (1, 50) = 4.74, $p < .05$, η^2 = .09] and in pNN50 ($p \leq .05$)

when considering the full sample of participants. No significant differences in self-regulation capacity were revealed between pain unpleasantness responders and non-responders.

In line with previous work, HRV measures were highly inter-correlated (Berntson et al., 1997, 2005; Task Force, 1996). No relationship was revealed between indices of vagal activation and sensory-discriminative or affective-motivational pain components.

Table 1. Descriptive and post hoc comparison values of pain rating measures[7]

	Mean	***SD***	***Minimum***	***Maximum***	***p*[a]**
All participants: *(N = 52):*					
Pain intensity	24.9	14.2	2.5	63.3	< .001**
Pain unpleasantness	19.6	14.9	0	64.2	< .001**
Pain intensity – Responders: *(n = 23)*					
Pain intensity	38.4	9.9	25.4	63.3	
Pain intensity – Non-Responders: *(n = 29):*					
Pain intensity	14.1	4.2	2.5	24.6	
Pain unpleasantness – Responders: *(n = 17):*					
Pain unpleasantness	36.1	11.5	25.8	64.2	
Pain unpleasantness – Non-Responders: *(n = 35):*					
Pain unpleasantness	11.6	8.2	0	23.8	

3.2.2. Logistic regressions

The computation of the predictive power of RSAnorm measures on pain-related sensations demonstrated that RSAnorm significantly influenced the LR model (see Table 3). The model [X^2 (1, N = 52) = 4.65, p < .05] explained between 8 % (Cox and Snell R square) and 11% (Cox and Snell R square) of the variation in the TGI responses. 75.9% responders and 52.2% non-responders were accurately identified (overall percentage: 65.4%). The RSAnorm-related high odds ratio value of 14.58 (CI: 1.12, 190.29) indicated that the probability to experience the illusive pain was 14 times higher in participants with more self-regulation ability than in those with less vagal activation.

[7] [a] Significance values of Mann-Whitney U tests: p-values < .05 (two-tailed) were considered significant.

Table 2. Descriptive and post hoc comparison values of resting HR and HRV indices[8]

	Mean	*SD*	*Minimum*	*Maximum*	*Group Comparison*	*p*[a]
All participants *(N = 52):*						
Mean HR (bpm)	71.9	10.4	50.2	95.3		
RMSSD	49.7	17.5	22.4	94.0		
pNN50	23.1	17.2	0	61.7		< .05*
HF (ms^2)	634.4	409.9	88.3	1976.2		
HF (n.u.)	42.2	19.6	7.9	84.9		
RSAnorm[b]	.80	.24	.35	1.37	*F* (1)=4.74	< .05*
Pain intensity – Responders *(n = 23):*						
Mean HR (bpm)	70.3	8.5	51.0	82.9		
RMSSD	52.7	16.7	23.2	85.3		
pNN50	28.2	16.5	2.2	59.6		< .05*
HF (ms^2)	731.4	357.2	88.3	1510.2		< .05*
HF (n.u.)	46.5	17.8	14.9	84.9		
RSAnorm	.88	.26	.35	1.37	*t* (27)= –2.53	< .05*
Pain intensity – Non-Responders *(n = 29):*						
Mean HR (bpm)	73.2	11.7	50.2	95.3		
RMSSD	47.4	18.1	22.4	94.0		
pNN50	19.1	16.9	0	61.7		< .05*
HF (ms^2)	572.1	442.72	129.2	1976.2		< .05*
HF (n.u.)	38.8	20.5	7.9	84.1		
RSAnorm	.74	.20	.45	1.19	*t* (27)= –2.53	< .05*
Pain unpleasantness Responders *(n = 17):*						
Mean HR (bpm)	69.5	9.2	51.1	82.9		
RMSSD	51.9	18.2	23.2	85.3		
pNN50	26.6	18.5	0	59.6		
HF (ms^2)	595.4	281.4	88.3	1094.8		
HF (n.u.)	46.0	17.6	14.9	84.1		
RSAnorm	.81	.22	.35	1.19		
Pain unpleasantness Non-Responders *(n = 35):*						
Mean HR (bpm)	73.1	10.8	50.2	95.3		
RMSSD	48.7	17.4	22.4	94.1		
pNN50	21.3	16.5	.4	61.7		
HF (ms^2)	649.8	462.7	129.25	1976.2		
HF (n.u.)	40.4	20.4	7.9	84.9		
RSAnorm	.80	.25	.45	1.37		

The LR analysis of the set of other HRV predictor variables showed that pNN50 and RMSSD contributed significantly to the model (see Table 3). The full model [X^2 (4, N =

[8] [a] Significance values of Mann-Whitney U Tests, independent t-tests, and One-way ANOVA: *p*-values < .05 (two-tailed) were considered significant and values < .001 (two-tailed) as highly significant. [b] RMSSD (square root of the mean squared differences of successive NN intervals), the proportion derived by dividing NN50 by the total number of NN intervals, high-frequency (HF, 0.15–0.4 Hz) values as expressed in power (ms^2) and normalized units (n.u.), Normalized respiratory sinus arrhythmia.

52) = 8.93, $p < .05$] explained between 15% (Cox and Snell R square) and 21% (Cox and Snell R square) of the variation in the pain responses. Overall 65.4% of the participants were accurately categorized either as pain responders (72.4%) or as non-responders (56.5%). The pNN50-related odds ratio was 1.16 (CI: 1.03, 1.31). The odds ratio of RMSSD was 0.88 (CI: .79, .99) indicating an inverse relationship between RMSSD and pain perceptions.

In summary, it may be stated that the resting HRV indices RSAnorm, pNN50, and HF (ms^2) were significantly higher in responders than in non-responders. Furthermore, the psychophysiological markers of dispositional self-regulation ability RSAnorm, pNN50, and RMSSD could be identified as predictors of the likelihood of paradoxical pain sensations.

Table 3: Predictors of thermal grill illusion perceptions[9]

	B	*S.E.*	*Wald*	*df*	*[a]p*	*Odds Ratio*	*95.0% C.I. for Odds Ratio*	
Predictors for pain intensity sensations:							*Lower*	*Upper*
Respiratory Sinus Arrythmia (RSA)	2.68	1.31	4.18	1	.04*	14.58	1.12	190.29
RMSSD	–.12	.06	4.42	2	.03*	.88	.79	.99
pNN50	.15	.06	6.38	2	.01*	1.16	1.03	1.31

4. Discussion

Previous research from our laboratory has revealed an involvement of psychological factors in thermal grill-related pain processing (Scheuren et al., 2014). The personality traits rumination and interoceptive accuracy as well as several interacting characteristics have been identified as enhancing the likelihood of the occurrence of the thermal grill illusion. Since a relationship between self-regulatory trait features and experimental or clinical pain processing has in addition been established (Appelhans & Luecken, 2006; Koval et al., 2013; Treister et al., 2012), these and our results suggested that self-regulation might also be involved in the commonly observed inter-individual differences in paradoxical pain perception (Boettger et al. 2011, 2013; Bouhassira et al. 2005, Lindstedt et al. 2011; Scheuren et al., 2014). In the present study, we hence explored

[9] [a]*p*-values < .05 (two-tailed) were considered significant.

differences in vagally mediated HRV at rest, which has frequently been used as a measure of self-regulation and psychological adaptability (Park et al., 2014; Park & Thayer, 2014; Thayer et al., 2009), to uncover whether higher or lower self-regulation capacity predicts pain sensitivity in response to the thermal grill paradigm. Our findings showed that individuals with a more pronounced extent of trait self-regulation ability were more likely to perceive the painful grill illusion. Affective-motivational pain responsiveness could not be predicted on the basis of self-regulation/ HRV levels.

To our knowledge, this is the first study investigating the relationship between a physiological indicator of self-regulatory capacity and the TGI. Self-regulation and HRV have so far only been explored in association with pain states depending on noxious input (Appelhans & Luecken, 2006; Koval et al., 2013; Solberg Nes et al. 2009; Treister et al., 2012). Our results do nevertheless not confirm our hypothesis of an inverse relationship between HRV and paradoxical pain. We had assumed that lower trait self-regulation ability would explain the higher pain sensitivity of the responders to the thermal grill paradigm. In the current study, more pronounced parasympathetically mediated HRV-indices like pNN50, HF (ms^2), and normalized RSA were however paired with more intense and frequent illusive pain sensations. The time domain components pNN50, RMSSD, and normalized RSA predicted the likelihood of an illusive pain occurrence. In this sense, the strong predictive power of RSAnorm (14 times higher probability of a TGI perception) corroborated the predominance of parasympathetic activity resp. self-regulatory capacity during the resting condition in the thermal grill responders. Concerning the RMSSD index of HRV, we observed that the low odds ratio result deviated to some extent from the other vagal activation indicator outcomes. However, although this time component highly but non-linearly correlates with pNN50, HF (ms^2), and RSAnorm (Berntson et al., 2005; Task Force, 1996), it has been considered not to fully represent cardiac vagal tone due to sympathetically mediated HRV contaminations (Berntson et al., 2005).

Unfortunately, there is a paucity of studies investigating the relationship between resting HRV or dispositional self-regulation and experimental or chronic pain. So far, Treister et al. (2012) reported a higher pre-stimulus (resting) HF (ms^2) value as compared to lower HF (ms^2) measured during subsequent noxious stimulation. Appelhans and Luecken (2008) disclosed that resting HF (ms^2) was related to the affective component of pain but not to sensory pain experiences. All other pain-related HRV depictions were of phasic nature and were recorded during acute painful stimulations (for review see Koenig et al., 2014). The phasic HRV-values mainly indicated that lower vagal reactivity was related to higher pain sensitivity. Solberg Nes and colleagues (2009) had analyzed the

relationship between trait self-regulation and pathological pain states and observed that chronic pain patients were characterized by lower self-regulatory ability than healthy individuals. On one hand, the scarcity of findings on self-regulation characteristics involved in classical pain processing hampers the attempt to offer explanations for the current outcomes. On the other hand, central neural mechanisms underlying the thermal grill have been claimed to be distinct from those involved in non-noxious *and* noxious thermal perceptions (Craig, 2008). This functional neuroanatomical aspect suggests that the regulating autonomous mechanisms acting during the TGI are not identical to those acting during pain processing induced by noxious thermal stimulation.

In a number of studies, higher HRV indices have been associated to more effortful and adaptive self-regulation, good impulse control, executive performance, lower affective instability and positive emotionality (Koval et al., 2013; Park et al., 2014; Park and Thayer, 2014), whereas lower HRV pointed to impaired coping processes, self-regulatory fatigue, stress, affective instability, and health-related problems like psychopathological disorders (Segerstrom & Solberg Nes, 2007; Solberg Nes et al., 2009). It has also been shown that participants with higher vagal activation react more easily when challenged by external demands (Rottenberg et al., 2005). The present results imply that individuals displaying a better trait self-regulation capacity, are affectively more stable, recover faster on an emotional level, and adapt more efficiently to challenging circumstances, are also more likely to react with heightened pain sensitivity at the thermal grill. Pain is well known to exert a warning function indicating a potential threat for tissue damage and for homeostasis and providing the drive for immediate protective and regulatory reactions (Craig, 2003). The efficient self-regulation of our thermal grill responders may hence constitute a healthy reaction allowing them to set their priorities successfully and to react faster and more adequately in the face of potentially threatening stimuli. The flexible adaptability of responders and the inherent efficient control of the emotional and behavioral drive of pain (Craig, 2003) promote their efficacy in reinstalling homeostasis. Pappens and colleagues (2014) have demonstrated that the maintenance of positive adaptation by safety learning or fear extinction will secure homeostasis in an even more efficient way.

In conclusion, the identification of an additional personality trait potentially involved in the regulation of paradoxical pain sensitivity may contribute to a better understanding of inter-individual differences in thermal grill-related pain perceptions. The disclosed pronounced level of vagally mediated HRV at rest in individuals perceiving the thermal grill illusion of pain provided evidence for their high prefrontally controlled emotional and cognitive self-regulation ability and their flexible adaptability to environmental

demands and homeostatic challenges. In order to allow for a more comprehensive comparison of previous findings on HRV-pain relationships with our results, the analysis of HRV recorded during the thermal grill stimuli will be required. The respective phasic cardiac measures would provide the possibility to compare effects of self-regulation capacity under conditions of stimulation and rest.

Studying relationships between self-regulation characteristics and thermal grill-induced sensations might furthermore be relevant for the identification of psychological factors contributing to neuropathic pain since thermal grill and central neuropathic pain mechanisms share common neural pathways.

Competing interests

The authors declare that they have no competing interests.

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4. General Discussion

The mind-body relationship is a recognized and highly interesting topic. Cognitive and related affective factors are known to have beneficial as well as detrimental effects on our body. In chronic pain, the harmful strong impact of adverse thoughts and emotions on nociceptive processing and pain has largely been documented. In health-related contexts and therapies, the mind-body connection is meanwhile firmly established and is integrated in cognitive-behavioural approaches to teach individuals how to use their thoughts for positive influences on the physical responses of the body. Hypnosis, relaxation techniques, and biofeedback for instance are examples of mind-body exercises that foster healing processes or provide for the control of stress.

Beneficial and adverse effects of psychological factors could also be retraced in the studies of the current thesis since the investigated cognitive and affective aspects were able to modulate pain sensations in both directions. On the one hand, learning processes in relationship with the HNCS-activated endogenous pain control system allowed the initially neutral phone signal to trigger similar psychophysical and psychophysiological responses as the tonic pain stimulus. It could be observed that pain rating and reflex levels related to phasic pain stimuli decreased in the post-conditioning phase. On the other hand, several psychological characteristics predicted increased pain sensitivity to thermal grill stimulations. It was shown that more ruminative, interoceptively accurate, and self-regulated individuals were more likely to perceive the painful grill illusion than those with less developed personality traits.

The activation of the pain-inhibits-pain mechanism following a differential respondent conditioning procedure is a novel finding that lends weight to the idea that we can learn to use mind-over-matter to beat pain. It had been shown that the brain could be conditioned to the ringtone being a signal to trigger the body's physical pain blocking mechanism. The people being tested not only felt significantly less pain, but there were also fewer objective signs of pain, such as activity in the muscles used in the facial expression of pain (frowning). In 2002, Flor and colleagues had already successfully conditioned the stress-induced analgesia mechanism. Both studies give evidence that associative learning processes are indeed capable to influence not only pain perceptions and behaviour, but also endogenous pain control mechanisms. Our results therefore corroborate the assumed involvement of descending pain modulation *and* learning processes in the observed long-lasting hypoalgesic effects of pain management techniques like transcutaneous electrical nerve stimulation (TENS), acupuncture or placebo treatments. It had previously been postulated that endogenous pain control mechanisms could not influence alone the

sustained pain improvement given that the duration of SIA- or DNIC-related effects fits the time course of drugs. HNCS-induced pain inhibiting effects measured in experimental settings are also of short duration and last about 10–15 minutes (Villanueva and Le Bars, 1995). It seemed conceivable that situational or environmental cues related to the pain treatment procedure (e.g. a white coat, the therapy-related environment) get associated to the pain alleviating effects of the therapy. The repeated pairing of this specific cue with pain relief might correspond to a conditioning procedure transforming the initially neutral stimulus in a conditioned stimulus. By remembering the CS, the hypoalgesic DNIC-like effects could be maintained over time and the observed time gap bridged (Carlsson, 2002; Price et al., 1984). On the basis of our results, it would be appealing to explore in a future research the duration of the measured CS-related effect. Could it be possible to show in a new HNCS-related pain model that the presentation of the CS an hour or a day after the experimental conditioning procedure allows retrieving a memory of the formerly conditioned stimulus and renew DNIC-like effects? With regard to the two phone signals used in the present study, this could mean that pain would at any moment get diminished when listening to or remembering the learned dial or busy phone signal. An intriguing perspective deserving further research efforts!

The painful sensations experienced at the thermal grill, the so-called 'thermal grill illusion of pain' or 'paradoxical pain' are interestingly considered as qualitatively different (i.e. paradoxical) from classically induced pain experiences and are only perceived by a fraction of the tested subjects (about one third in average; Bouhassira et al., 2005; Boettger et al., 2013). A further point is that the central mechanisms of the TGI are discussed as being distinct from the mechanisms underlying non-noxious and noxious thermal sensations (Craig, 2008). However, the same distinct neural circuitry plays a role in the dysfunctional interaction between temperature and pain in central neuropathic pain conditions. For this reason, the thermal grill has recurrently been used as a tool to unravel the neurophysiological basis for the thermal grill–central pain interaction and the dysfunctional thermosensory integration in neuropathic pain patients (Craig and Bushnell, 1994; Craig et al., 1996, 2000; Kern et al., 2008; Lindstedt et al., 2011).

The lack of explanations for the described inter-individual differences in susceptibility to the expression of paradoxical pain and the missing data on psychological characteristics potentially underlying thermal grill-related pain processing incited us to conduct the two thermal grill studies. The protocol was inspired by the relationship between pain-related personality traits or parameters like IA and self-regulation capacity and pain expression uncovered in clinical or experimental pain models involving suprathreshold *noxious* stimulations (Pollatos et al., 2012; Sullivan et al., 2005; Tang and Gibson, 2005; Wiech et al., 2008). In our thermal grill paradigm, the traits were analysed

in association with painful sensations induced *without* the presentation of any noxious input. The aim of the studies consisted in finding out whether the pain-enhancing characteristics identified in classical pain research also acted in association with the innocuous thermal grill stimuli and explained the variance in paradoxical pain sensitivity. Since depression and state mood had already been investigated in association with thermal grill-related pain sensitivity and central pain processing (Boettger et al., 2011; Piñerua-Shuhaibar et al., 2011), we did not focus on these psychological aspects in our more explorative analysis.

Our findings revealed that some of the assessed personality traits were strongly related to paradoxical pain perceptions. Especially rumination and IA significantly increased the likelihood of thermal grill-induced pain experiences. Uncovered interaction terms gave further insight in synergistic cognitive and emotional mechanisms that are essential for the TGI experience. Several findings were different from those observed in classical pain research. In these studies relying on noxious input, the cognitive factor rumination for instance only adversely influenced pain perceptions when considered as a sub-factor of pain catastrophizing (Sullivan et al., 1995). In the present context however, rumination was not meaningfully related to pain catastrophizing, but significantly predicted the TGI occurrence when considered alone. Also, an interaction between rumination and IA has not been identified in previous pain research. The combination uncovered in the present study suggests that rumination-related negative cognitions of responders and the extent of IA, as a measure for the sensitivity to somatic signals and an indicator of the intensity of emotional processing, may interdepend in the sense that perseverating negative thoughts and concomitant intense emotions may wind each other up and by this way exacerbate paradoxical pain sensitivity. As concerns anxiety, pain expectancy, and pessimism, these factors have so far been mainly related to pain catastrophizing and not to the catastrophizing sub-factor rumination (Sullivan et al., 2005). Generally seen, our interaction results stipulate that the occurrence of the TGI seems to depend on affective influences (e.g. state anxiety, interoceptive accuracy) that are maintained by cognitive factors like perseverative thoughts, expectancies, or dispositional pessimism. With regard to the pain unpleasantness-related results, it was shown that beside rumination, suggestibility aspects played a role in the prediction of the elicitation of the affective-motivational component of paradoxical pain. None of the other analysed personality traits influenced the unpleasant experience of the TGI. These findings again contrast classical pain research outcomes and should be kept in mind in further thermal grill studies. The detected dissimilarities possibly underline the observed difference between central neural mechanisms underlying the TGI and those related to non-noxious and noxious thermal stimuli.

The temperature combination of 15°C and 41°C used in our experimental condition was taking into account other thermal grill studies establishing that larger temperature differences between the cold and warm bars of a thermal grill (i.e. about 21–26 °C) safely allowed eliciting the TGI and yielded higher pain ratings (Bouhassira et al., 2005; Boettger et al., 2011, 2013). Bouhassira and colleagues e.g. reported that "in about one third of the sample the phenomenon was observed only with the largest differentials of temperature between the warm and cold bars". In the control conditions, we combined the baseline temperature of 32°C with the experimental temperature of 15°C (superior to CPT) respectively 41°C (inferior to HPT). With regard to the circumstance that the TGI characteristically results from a combination of explicit cold and warm stimuli and the underlying parallel stimulation of cold and warm receptors, a 32°C temperature (also ± mean temperature measured at the hands of our participants) is typically not considered as a warm noxious stimulation, but as a neutral or indifference temperature (i.e. the baseline body surface temperature measured on average under conditions of normal ambient room temperature) that is not capable to induce a TGI.

The absence of a common definition of responders and non-responders to grill stimuli hampered to some extent the classification of both groups of participants. While separating the sample on the basis of their pain intensity and pain unpleasantness ratings, we used the same identification methods as those applied in former grill studies (Bouhassira et al., 2005, Boettger et al., 2011, 2013) to discriminate between subjects perceiving or not perceiving the TGI. We suggest to aspire in the future for an agreement on a generally accepted definition, all the more since painful sensations measured in previous thermal grill studies were associated with a large variety of temperature combinations or rating scales.

In our second grill study, vagally mediated heart rate variability has been assessed during a pre-experimental resting condition to define the extent of dispositional self-regulation capacity in the participants. The responders were characterized by higher self-regulatory ability than the non-responders. This personality aspect, as mainly emphasised by the HRV-index RSA, predicted the likelihood of paradoxical pain sensations in response to the grill stimuli. It was concluded that the affectively more stable responders, with faster emotional recovering, and more adaptive ability in challenging circumstances or homeostatic demands, are also more likely to react with enhanced pain sensitivity at the thermal grill. The warning function of pain signaling an acute threat for tissue damage and for homeostasis and providing the drive for immediate protective and regulatory reactions has been extensively described (Craig, 2003). All in all, the current finding suggests that the efficient self-regulation of the responders constitutes a healthy reaction that allowed them to set their priorities successfully and to react faster and more adequately when facing

the potentially threatening pain stimulus in the following experimental condition. It may be assumed that during the grill stimulation phase, they most probably adapted in a flexible way and efficiently controlled the emotional and behavioural drive of pain (Craig, 2003), thus promoting the re-establishment of homeostasis.

The revealed interaction between higher interoceptive sensitivity, increased pain perceptions (in experimental pain model with noxious input) and lower vagal activation (Pollatos et al., 2012) possibly points to the pertinence of the discussion of our IA-related result in association with higher paradoxical pain sensitivity (cf. study 2 – Scheuren et al., 2014) and greater resting HRV. When furthermore considering that other studies have related the improved detection of somatic signals to more intense emotional experience and processing (Damasio, 1999; Herbert et al., 2007, 2010), it is conceivable that the enhanced IA and emotionality of our responders was responsible for higher physiological arousal (e.g. blood pressure) and accentuated attention to the thermal grill stimulation. As a consequence, these influences may have emphasized the relevance of the thermal stimulus and the sensitivity to it. The influence of their dispositional self-regulation capacity probably allowed the respective subjects to cope in an effortful (Park et al., 2014) and successful way (Solberg Nes et al., 2009) with the painful challenge and to reinstall homeostasis while controlling for the arousing cardiac and emotional perceptions.

To further elucidate the reasons for the enhanced pain sensitivity of the responders and to test our hypothesis, we propose to analyse in a next step the HRV-data that we collected *during* the experimental stimulation phase of the present study. These phasic HRV-results would define the self-regulatory strength of thermal grill responders during acute pain processing. Although time limits prevented these analyses so far, it will be interesting to uncover whether self-regulatory capacity of the responders warranted efficient regulation in the experimental phase or whether their capacity was also lowered during the painful perceptions as has been observed in classical experimental pain research or clinical pain conditions.

5. Future perspectives

The identification of psychological mechanisms affecting noxiously and non-noxiously elicited pain processing provides new and interesting insight on the impact of affective and cognitive processes on pain modulatory mechanisms and related pain perceptions. It has to be analysed whether the uncovered influences may also underlie pathological pain conditions so as to foster the development of new psychological treatment strategies.

In the framework of the relationship between learning processes and endogenous pain modulation, the generation and investigation of long-lasting pain inhibitory effects has already been initiated in our laboratory. Unfortunately, the study could not be brought to an end because of technical problems. Another experimental attempt should therefore be started to define the parameters necessary for optimizing the new paradigm and to provide evidence for long lasting pain inhibitory effects. New pain treatment approaches on the basis of respondent conditioning procedures with environmental or situational cues that are familiar to a patient might in consequence be targeted.

The findings of the innocuously-based thermal grill studies are important in this field of research in the sense that the revelation of the influence of dispositional self-regulation capacity, rumination, interoceptive accuracy, and their interacting mechanisms on the likelihood of paradoxical pain perceptions underlines the importance of those factors in the understanding and treatment of the dysfunctional interactions between thermo-sensory and nociceptive processing as observed in central neuropathic pain patients. The examination of these significant characteristics may also be seen as an important step in the further elucidation of e.g. psychosomatic pain complaints or pain conditions with medically unexplained symptoms. Gender might be another interesting factor in future thermal grill research that could illuminate sex-related differences in the pathological pain states of interest. An additional research perspective may be seen in the comparison of thermal grill and contact thermode-related thermal stimulation outcomes. The assessment of the magnitude of psychophysical and HRV-values measured under both experimental conditions might reveal interesting differences in pain ratings and self-regulation strength depending on the respective (non-noxious or noxious) thermal stimulation.

6. Literature

6.1 Introduction

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