Cognitive and Behavioral Context of Pain Facilitation - Nocebo Conditioning and Uncontrollability-Induced Sensitization

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Preface

The studies presented in this thesis were conducted at the Otto Selz Institute for Applied Psychology at the University of Mannheim (Studies 1 and 2) and at the Alan Edwards Centre for Research on Pain (AECRP) at McGill University in Montreal, Canada (Study 3), during a research visit. Chapters 2.1 and 3 are intended for publication in modified form. Chapter 3 was developed in collaboration with Prof. Dr. Petra Schweinhardt. Prof. Dr. Kleinböhl developed a routine for the transformation of the raw heart rate data (Study 2) and contributed in the technical implementation of Study 3, amongst others.

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Abbreviations

ACC	anterior cingulate cortex
CCK	cholecystokinin
CR	conditioned response
CS	conditioned stimulus
DNICS	diffuse noxious inhibitory controls
fMRI	functional magnetic resonance imaging
HSA	hypothalamic-sympathetic-adrenal
JND	just noticeable difference
LEPs	laser-evoked potentials
LMM	linear mixed model
PAG	periaqueductal grey
PASS	Pain Anxiety Symptom Scale
PCA	patient-controlled analgesia
PFC	prefrontal cortex
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
STAI	State-Trait Anxiety Inventory
rTMS	repetitive transcranial magnetic stimulation
UR	unconditioned reaction
US	unconditioned stimulus
VAS	visual analog scale

1. General Introduction

According to the International Association for the Study of Pain (IASP), pain is "an unpleasant sensory and emotional experience, associated with actual or potential tissue damage or described in terms of such damage" (Merskey & Bogduk, 1994). It is a subjective sensory and emotional experience, susceptible to the influence of various pain-modulating factors (McGrath, 1994; Staats, Hekmat, & Staats, 1996; Tracey, 2008), like the environment (Abbott, Franklin, & Connell, 1986; Malenbaum, Keefe, Williams, Ulrich, & Somers, 2008), learning (Becker, Kleinböhl, Baus, & Hölzl, 2011; Flor, Birbaumer, Hermann, Ziegler, & Patrick, 2002), attention (Bantick et al., 2002; Valet et al., 2004), emotion (Roy, Piche, Chen, Peretz, & Rainville, 2009; Villemure, Slotnick, & Bushnell, 2003), cognition (Seminowicz & Davis, 2006; Weissman-Fogel, Sprecher, & Pud, 2008), and contextual factors (Moseley & Arntz, 2007). The pain experience usually depends on noxious stimulation, peripheral nociceptive activity, and central processing and can be modulated already at low levels of the nervous system (Wall, Melzack, & Bonica, 1999), such as in peripheral sensitization (Woolf & Salter, 2000) as well as in later stages of the pain processing cascade (Lorenz, Minoshima, & Casey, 2003). Extreme examples that illustrate the complex interplay between the various components involved in pain processing are phantom pain and a rare condition called congenital insensitivity to pain (CIP). In phantom pain, a pain experience is evoked without direct (peripheral) nociceptive input of the apparently affected location, even without existence of the pain-causing limb. Although in many instances the stump receives nociceptive input, for example due to a stump neuroma, studies suggest that this subjective pain perception—despite missing counterpart in the body's outer appearance—depends in part on reorganization processes in somatosensory cortical areas (Flor, Nikolajsen, & Staehelin Jensen, 2006; Karl, Birbaumer, Lutzenberger, Cohen, & Flor, 2001). Interestingly, mirror therapy seems to reduce phantom pain in many cases, which can be attributed to somatosensory learning processes and corresponding cortical restructuring (MacLachlan, McDonald, & Waloch, 2004). In contrast, patients with CIP do not feel physical pain at all, probably due to a genetic abnormality that causes malfunctioning nociceptors (Nilsen et al., 2009). These patients often suffer from severe injuries, especially in their childhood, because their body's warning system is amiss. They have to learn consciously and with great effort what comes naturally to unaffected people, for instance not to touch a hotplate, because they lack the

flexor reflex normally causing a defensive reaction. Both phantom pain and CIP illustrate that pain is a complex phenomenon, depending on the interplay between numerous components. In healthy subjects, it helps to protect the body against injury and harm by incorporating various external and internal factors of influence, thereby remaining flexible and plastic.

As the success of interventions like mirror therapy demonstrates, the pain experience can be altered and influenced by psychological factors. Besides deliberate therapeutic strategies, pain modulation can, and indeed does, most of the time occur without the subject being aware of it. A wound, for example, will be less bothersome as soon as a patient is distracted. Factors of influence can either be inhibitory (e.g., placebo, distraction, stress-induced analgesia, sense of control) or facilitatory (e.g., attention, nocebo, catastrophizing thoughts). Compared to pain-inhibiting factors, pain facilitation is less studied, although it is possibly an important determinant in chronic pain (Turk & Okifuji, 2002). In this thesis, exemplarily for different psychological factors potentially involved in pain facilitation, the specific cases of conditioning-induced nocebo effect and uncontrollability are investigated. Both are supposed to be important factors in the context of chronic pain and have to be dealt with in clinical routine. Although different mechanisms are at work, evidence suggests that nocebos and uncontrollability can lead to increased fear (Bingel et al., 2011; Crombez, Eccleston, De Vlieger, Van Damme, & De Clercq, 2008) and feelings of helplessness (Müller, 2011; Vogtle, Barke, & Kroner-Herwig, 2013), contributing to a self-maintaining vicious circle of pain that potentially activates the descending pain modulatory system.

1.1 Placebo and Nocebo Effects

The significance of the placebo effect (i.e., a desirable effect after an inert treatment) is widely recognized: One can take advantage of it in clinical situations (e.g., lowering the dosage of medication) and it plays a major role in the conduction of pharmaceutical studies, for example, when subjects in the placebo group show major improvements although the treatment is inert. The nocebo effect (i.e., an undesirable effect after an inert treatment), although less well-understood, significantly affects clinical situations, as well: It can cause, for example, deterioration of symptoms in adverse physician-patient support or when a patient is diagnosed with a severe disease. Extreme examples are cases of

apparently life threatening events ("Beinahe-Tod") as a consequence of voodoo magic, acting as a nocebo (Cannon, 2002). Research concerning the nocebo effect can shed light on the occurrence of side effects (Kaptchuk et al., 2006), processes in acute pain, and factors contributing to chronic pain, because the development and maintenance of chronic pain are affected by psychological factors, as outlined above.

More precisely, a placebo (Latin: "I shall please"), according to Stewart-Williams & Podd (2004), is "a substance or procedure that has no inherent power to produce an effect that is sought or expected" (p. 326). The placebo effect is "a genuine psychological or physiological effect, in a human or another animal, which is attributable to receiving a substance or undergoing a procedure, but is not due to the inherent powers of that substance or procedure" (p. 326). This definition does not make assumptions about the desirability of the effects and therefore includes what is often labeled the 'nocebo effect', i.e., a 'negative' placebo (for example pain *increase* instead of pain *relief*). Incorporating both phenomena in a single expression avoids some pitfalls (e.g., some placebos might show desirable and undesirable effects at the same time or different subjects might interpret the same effect oppositional). Yet, explicitly distinguishing between placebo and nocebo (Latin: "I shall harm") and respectively placebo and nocebo effects seems to be essential because evidence suggests that in part, different principles apply (Colloca, Sigaudo, & Benedetti, 2008). The expression 'nocebo effect' stresses aversive consequences and is defined by Benedetti, Lanotte, Lopiano, & Colloca (2007) as "a phenomenon whereby anticipation and expectation of a negative outcome may induce the worsening of a symptom" (p. 260).

Psychological mechanisms of placebo and nocebo effects

Current research explains placebo and nocebo effects across different systems (nociceptive, immune, motor, etc.) mainly by means of two mechanisms: classical conditioning and expectancy (Stewart-Williams & Podd, 2004). It is commonly accepted that these mechanisms can cause placebo and nocebo effects both independently (i.e., only by expectation) and in combination, depending on the system or disease in question. Recent evidence further suggests that observational learning is capable to cause placebo (Colloca & Benedetti, 2009) and nocebo effects (Vogtle et al., 2013). Especially in the context of pain, the exact interrelations and conditions that cause placebo and nocebo effects are far from being clearly established, yet. For example the significance of conditioning remains

controversial for the nocebo effect and whether learning is effective if it occurs without awareness (like in implicit conditioning) is not known, yet. However, precise knowledge of the exact mechanisms of placebo and nocebo effects in pain perception is necessary, e.g., to control for the effects in pharmaceutical studies and to maximize the benefit in a therapeutic context. When the conditions under which the placebo and nocebo effect are shaped by conditioning and/or expectancy are understood clearly, the treatment can be adopted accordingly. If applicable, one can consider and integrate laws of conditioning, like generalization, blocking, latent inhibition, etcetera. For example, if latent inhibition applies, it is probable that an ineffective treatment can have negative consequences for a subsequent treatment attempt. In cases, in which expectation is considered very important, a consequence for the treatment could be to carefully consider the choice of words or to take extra time for explaining the expected positive effects.

The nocebo effect and its mechanisms are less well explored compared to its more desirable counterpart (Kong et al., 2008). There is evidence that a nocebo effect is not just a reversed placebo effect, although probably the same basic mechanisms as for the placebo effect apply to the nocebo effect (Benedetti, Amanzio, Vighetti, & Asteggiano, 2006; Colloca et al., 2008; Kong et al., 2008). For instance, an additional learning procedure, compared to conscious expectation by verbal suggestions only, did not lead to further enhancement of the nocebo effect, but it led to an increased placebo effect (Colloca et al., 2008). Further, in the research of placebo and nocebo effects it should be noted that those effects are not uniform constructs, but differ depending on different systems and diseases (e.g., pain relief, improvement of motor functioning, allergic reactions, depression; Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Benedetti, 2008). Different placebo and nocebo effects are probably even caused by various mechanisms.

According to expectancy theory, a placebo/nocebo effect occurs because a placebo/nocebo induces a specific expectation (Stewart-Williams & Podd, 2004). It is worth noting that expectancy, here, is understood as consciously accessible (in humans: reportable). In the experimental setting, expectations are mostly induced by verbal suggestions (e.g., the experimenter explains to the subject that a powerful analgesic is applied). However, the administration of a placebo/nocebo in form of an ointment or a tablet (without any additional verbal suggestion) can already induce expectations. Further, expectancy theory only refers to verbally reportable expectations and therefore does not take into account the occurrence of expectancies without awareness (e.g., measurable through anticipatory physiological parameters). Unconscious (or implicit) expectancies (“beliefs”, Haug,

2011), however, refer to content that is potentially consciously accessible, for example when attention is drawn to it (Haug, 2011).

In classical conditioning, a formerly neutral stimulus, for example a bell, becomes a conditioned stimulus (CS), capable of triggering a specific response when it is repeatedly coupled to an unconditioned stimulus (US), e.g., food that evokes an unconditioned response (UR), like salivation. Subsequently, the presentation of the CS alone leads to a response (conditioned response, CR), that is similar to the UR (but can differ in latency, amplitude and configuration; Hilgard, 1936). In most cases, explicit classical conditioning takes place, meaning that during the procedure the subjects become aware of the contingencies between CS and US. When the subjects can report this relationship, an expectation has evolved as an epiphenomenon of the conditioning (Kirsch, 2004). However, classical conditioning can also occur implicitly, i.e., without the subject being aware of the contingencies between CS and US (Clark, Manns, & Squire, 2002; Manns, Clark, & Squire, 2001; Manns, Clark, & Squire, 2002, for review). In these instances, the development of a conscious expectation can be excluded.

Conditioning emphasizes the importance of learning through direct experience (Voudouris, Peck, & Coleman, 1990). When, in the specific context of pain, the placebo effect is explained in terms of classical conditioning (Wickramasekera, 1980), an active drug or procedure (e.g., an analgesic) serves as unconditioned stimulus leading to pain relief (UR). The active agent might be administered in form of a pill, so that the pill serves as CS. After repeated simultaneous presentation, the pill itself, lacking an active agent (and therefore representing a placebo), leads to pain relief (CR). This conditioned response represents the placebo effect. In cases in which the drug or procedure leads to aversive consequences, such as a burning sensation, a nocebo effect was induced. The role of conditioning in the placebo and nocebo context is supported by animal studies, as the concept of conscious expectation does not apply here. Examples for conditioning-induced nocebo effects in animal models are immunosuppression after illness-induced taste aversion in the rat (Ader & Cohen, 1975) and disruption of learned behavior by saline injection after conditioning with a suppressive drug (Herrnstein, 1962). Experiments demonstrated that typical characteristics of conditioning (e.g., stronger US produce stronger CR, i.e., dose-dependency, extinction) apply (Gliedman, Gantt, & Teitelbaum, 1957).

Both expectancy and conditioning can induce physiological (objectively measurable) and subjective placebo and nocebo effects (Benedetti et al., 2006;

Colloca & Benedetti, 2006; Colloca et al., 2008; Colloca et al., 2009). Evidence suggests that conditioning causes stronger (Voudouris et al., 1990) and longer lasting placebo effects than verbal suggestions (Klinger, Soost, Flor, & Worm, 2007). In the context of pain, interestingly, placebo-hypoalgesia that was induced by conditioning can be mediated by various neurobiological mechanisms (Amanzio & Benedetti, 1999): When the subjects were conditioned with a non-opioidergic analgesic (e.g., ketorolac), the conditioned response was mediated by non-opioid mechanisms (naloxone-insensitive), whereas when the subjects were conditioned with an opioidergic analgesic, like morphine, the conditioned response relied on opioidergic mechanisms (naloxone-reversible). In contrast, expectancy-induced hypoalgesia seemed to depend solely on opioidergic mechanisms (naloxone-reversible). Further studies found evidence for a positive relation between the individual placebo response and dopamine release (Scott et al., 2007). Here, positive expectations were interpreted as a special case of reward anticipation (Petrovic et al., 2005). These results support the notion that expectation- and conditioning-induced placebo effects are not dependent upon identical mechanisms. However, studies that exclusively employ a conditioning procedure to induce placebo or nocebo effects allowing to separate the effects of both mechanisms are scarce.

According to Benedetti et al. (2003), only immune and hormonal placebo responses can be caused by conditioning. Placebo effects in other systems, like the nociceptive system, are supposed to be mediated necessarily by conscious expectations, which can be formed through conditioning or verbal suggestions (Price, Finniss, & Benedetti, 2008). Stewart-Williams & Podd (2004), on the other hand, come to the conclusion that placebo and nocebo effects can occur without expectancy only when classical conditioning is mediated through pharmacological agents that provoke physiological changes, which are not consciously perceived by the subjects. For example, Benedetti et al. (1998) and Benedetti, Amanzio, Baldi, Casadio, & Maggi (1999) induced placebo respiratory effects that resulted from buprenorphine conditioning and were not noticed by the subjects. Both Stewart-Williams & Podd (2004) and Benedetti et al. (2003) concur in stating that conditioning only and exclusively is sought to cause a placebo effect (without expectancy) when this effect is unaware (Benedetti et al., 2003: immune or hormonal changes; Stewart-Williams & Podd, 2004: pharmacological effects). However, it is not clear why conditioning without the induction of conscious expectations should not be sufficient to induce placebo and nocebo effects in the

nociceptive system. Further, the role of contingency awareness has not been investigated in the context of placebo and nocebo conditioning.

Assessment of placebo and nocebo effects

In past research, placebo and nocebo effects have almost always been studied by means of subjective ratings (e.g., numeric pain ratings; Benedetti et al., 2003; Colloca & Benedetti, 2006; Colloca et al., 2008; Colloca & Benedetti, 2009; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010). However, placebo and nocebo manipulations (e.g., verbal suggestions of pain increase) do not necessarily result in subjectively altered pain ratings (Johansen, Brox, & Flaten, 2003; Vogtle et al., 2013). There are only few studies that implemented other assessment methods, like behavioral measures (e.g., pain tolerance in the tourniquet test, Amanzio & Benedetti, 1999; velocity of movement in patients with Parkinson's disease, Benedetti et al., 2003). Goffaux, Redmond, Rainville, & Marchand (2007), for example, found increased withdrawal reflexes after hyperalgesia compared to hypoalgesia suggestions in a DNICS (diffuse noxious inhibitory controls) paradigm. Further, psychophysiological measures assessing autonomic changes are rather rarely studied and results are inconsistent, probably varying with the outcome variable and the experimental paradigm. For example, after placebo manipulation, de Jong, van Baast, Arntz, & Merckelbach (1996) did not find conditioned skin conductance responses despite pain decreases on a subjective level. In another study by Pollo, Vighetti, Rainero, & Benedetti (2003), placebo administration accompanied by a verbal instruction caused reduced heart rate and sympathetic responses. Contrary to that, Matre, Casey, & Knardahl (2006) demonstrated reduced heat pain sensitivity and smaller hyperalgesic and allodynic areas but no effect on heart rate and blood pressure after placebo manipulation with a sham magnet. Kirsch & Weixel (1988) showed expected effects on pulse rate and systolic blood pressure in a deception but not in a double-blind placebo administration. In another study, a dissociation between different measures was found: Following a conditioning procedure, but not a verbal suggestion, subjective pain ratings decreased. However, compared to a control group, laser-evoked potentials (LEPs) were strongly decreased in the conditioning group and to a weaker degree also decreased in the verbal suggestion group. This dissociation between subjective rating and LEP suggests that a certain threshold might have to be reached until the placebo effect is detectable in conscious perception or in the subjective rating (Colloca et al., 2009).

In summary, most studies assess placebo and nocebo effects with subjective ratings. Although evidence shows that subjective ratings cannot comprehensively capture and represent the changes induced by a placebo or nocebo, especially for the nocebo effects only few studies exist that investigate psychophysiological and behavioral measures.

Nocebo effects in pain perception

In the context of pain, a nocebo leads to increased pain sensitivity (nocebo-hyperalgesia), which can be assessed, for instance, by means of decreased pain threshold or increased pain sensation. Although far less is known about the mechanisms of nocebo-hyperalgesia, literature suggests that probably the same basic mechanisms as for the placebo effect (i.e., expectancy, conditioning) apply to the nocebo effect (Colloca et al., 2008).

Expectation-induced nocebo effects: It is assumed that verbally induced negative expectations (e.g., "This procedure will lead to increased pain sensitivity.") induce anticipatory anxiety about the impeding pain. This anxiety (indicated by hypothalamic-sympathetic-adrenal (HSA) hyperactivity) is sought to activate cholecystokinin (CKK), a neuromodulator implicated in pain modulation and anxiety (Hebb, Poulin, Roach, Zacharko, & Drolet, 2005). Whereas both HSA hyperactivity and hyperalgesia were blocked with benzodiazepines, suggesting the involvement of anxiety in the formation of the hyperalgesia, the CCK-antagonist proglumide proved to antagonize only the nocebo-hyperalgesia, leaving anxiety-related HSA hyperactivity unaffected. Thus anxiety-triggered CCK activation was assumed to facilitate the pain transmission, finally resulting in hyperalgesia (Benedetti & Amanzio, 1997; Benedetti, Amanzio, Casadio, Oliaro, & Maggi, 1997; Benedetti et al., 2006; Colloca & Benedetti, 2007, for review).

Conditioning-induced nocebo effects: Based on results from animal studies, Voudouris, Peck, & Coleman (1985) introduced a conditioning design for humans in order to induce placebo and nocebo effects serving as model for many subsequent conditioning studies. They applied an inert cream along with a suggestion of pain relief and surreptitiously increased (nocebo manipulation) or decreased (placebo manipulation) stimulus intensities on trials with cream compared to trials without cream and observed changes in pain ratings in the direction according to the manipulation. In an analogous experiment, they further demonstrated that conditioned nocebo, but not placebo responses generalized

from an iontophoretic to an ischemic pain model (Voudouris, Peck, & Coleman, 1989).

Colloca et al. (2008) directly compared placebo-hypoalgesia and nocebo-hyperalgesia either when only a verbal suggestion was given, or an additional conditioning procedure was conducted before the verbal suggestion. For the placebo effect, conditioning played a major role because verbal suggestions alone led to less pain decrease compared to the combination of conditioning and verbal suggestions (cf. Voudouris et al., 1990). For the nocebo effect, however, additional conditioning did not lead to further increases in the pain ratings compared to verbal suggestions alone. The authors concluded that learning is less important in nocebo-hyperalgesia compared to placebo-hypoalgesia (Colloca et al., 2008; Petrovic, 2008). However, this conclusion appears to be premature because the effect of conditioning was only investigated in combination with a verbal suggestion. In order to truly evaluate the impact of conditioning on nocebo effects, studies need to induce nocebo-hyperalgesia by conditioning alone. Only one study so far, induced a nocebo effect by conditioning alone, without giving additional verbal suggestions or other cues (e.g., pills) that potentially induce expectations from the outset (Jensen et al., 2012). The results demonstrated that nocebo-hyperalgesia could be successfully induced after conditioning. A nocebo effect even occurred when the facial stimuli, serving as CS, were presented subliminally, indicating that nocebo effects can be activated by perceptions that stay beyond the level of consciousness. However, during conditioning, the CS were presented supraliminal and contingency awareness was not assessed. Thus, whether a nocebo effect can be induced by implicit conditioning as well remains to be determined.

To summarize, according to previous research, the placebo effect in the context of pain can be established through expectation or conditioning. But when it is established by conditioning, it is sought to be mediated by expectation that developed during the conditioning procedure. The nocebo effect is mainly assumed to depend on expectation, although in a recent study, nocebo-hyperalgesia could be activated by subliminally presented cues after an explicit conditioning procedure (Jensen et al., 2012).

1.2 Uncontrollability and Pain Perception

An important factor influencing the reception of aversive events and especially the pain perception is somebody's sense of control. Although Study 3 of this thesis is concerned with the effect of uncontrollability (i.e., lack of control) on painful stimulation, most research has focused on the impact of *having* control rather than *not* having it.

Control can be defined as "some behavior (overt or covert) that reliably changes something else" (Arntz & Schmidt, 1989). Here, the subjective perception of control is most essential, which becomes obvious in Thompson's (1981) definition, who puts control as the "belief that one has at one's disposal a response that can influence the aversiveness of an event" (p. 89). The notion that controllability affects the aversiveness of an event, or, specifically in the context of pain, the painfulness, seems self-evident and in concordance with our personal experience. Accordingly, especially chronic pain patients seek control for their conditions. However, reviews on this topic do not always entirely agree. Whereas Averill (1973) and Thompson (1981) come to the conclusion that controllability does not reliably decrease the impact of aversive events, Miller (1979), who investigated effects of *behavioral* control, concluded that controllable aversive events may have less negative effects. Finally, Arntz & Schmidt (1989) restricted their analysis to the control of noxious, painful stimuli and summarized that perceived control can reduce negative effects of pain, depending on the outcome measure (subjective, behavioral, physiological), type of control, and salience.

In the context of control, many related concepts play a role, for example learned helplessness, and coping. Learned helplessness (Abramson, Seligman, & Teasdale, 1978) "refers to a constellation of behavioral changes that follow exposure to stressors that are not controllable by means of behavioral responses, but that fail to occur if the stressor is controllable" (p. 829, Maier & Watkins, 2005). It can result in motivational, cognitive, and learning deficits (Seligman & Maier, 1967; Seligman, Rosellini, & Kozak, 1975, cited after Abramson et al. 1978) and serves as a model for clinical depression (Abramson, Metalsky, & Alloy, 1989). Coping is understood as "purposeful efforts to manage or vitiate the negative impact of stress" (p. 250, Jensen, Turner, Romano, & Karoly, 1991). In the context of pain, it is defined as efforts, usually involving cognitive and behavioral strategies, to cope with, deal with, and minimize pain and pain-related distress and disability (Rosenstiel & Keefe, 1983). A coping strategy can consist of relaxation, distraction,

positive self-statements, imagery strategies, hypnosis, stress inoculation training, cognitive transformation of the situation, etc. (Arntz & Schmidt, 1989). Coping self-statements and reinterpretation of pain sensations predict greater perceived control, as well as flexibility in coping (Haythornthwaite, Menefee, Heinberg, & Clark, 1998).

Typology and effects of control

Control can be distinguished into different types, which potentially have differential effects on the painfulness of events. Averill (1973; also refer to Thompson, 1981) suggested a classification into *behavioral control* (the availability of a response that may directly influence or modify the objective characteristics of a threatening event; either regulated administration or stimulus modification), *cognitive control* (the processing of potentially threatening material in such a way as to reduce the net long-term stress or psychic costs of adaption, e.g., reappraisal), and *decisional control* (the opportunity to choose among various courses of action). Miller (1979) further dissected behavioral control into *instrumental control* (the ability to make a behavioral response that modifies the aversive event), *self-administration* (the self-delivery of the aversive event), *actual control equated for predictability* (controllability and predictability are kept methodologically distinct), and *potential control* (the person believes that some controlling response is available but is not actually used). Finally, Arntz & Schmidt (1989) expanded this list with the concept of *loss of control* and thereby for the first time explicitly focused on uncontrollability (cf. Staub, Tursky, & Schwartz, 1971). In this thesis (Study 3), subjects are given instrumental control over the intensity of a temperature stimulus before their task changes and they lose control over the temperature input.

Evidence shows that *behavioral control* most reliably affects the painfulness of an event (Arntz & Schmidt, 1989, for review). It increases pain tolerance (Bowers, 1968; Litt, 1988), affects physiological measures (heart rate, Weisenberg, Wolf, Mittwoch, Mikulincer, & Aviram, 1985; skin conductance response, Corah & Boffa, 1970), and decreases subjective pain report (Borckardt et al., 2011; Bowers, 1968; Weisenberg et al., 1985). Uncontrollable painful stimulation, on the other side, increases perceived pain intensity (Müller, 2012; Wiech et al., 2006) and cortisol secretion as an indicator of the stress response (Müller, 2011) and leads to decreased pain tolerance (Staub et al., 1971). Losing control, compared to never having had control at all, leads to a more unpleasant pain experience, increased

fear, heightened vigilance to the pain sensation, and impaired post-exposure performance (Crombez et al., 2008). Repeated failing attempts to control pain resulted in increased anger and heart rate responses (Janssen, Spinhoven, & Arntz, 2004).

Psychological and neural mechanisms of (un)controllability

Different theories exist that try to establish how control influences the pain experience. Whereas Bowers (1968) hypothesized that *anxiety* mediates the level of pain in case of perceived lack of control (Wiech et al., 2006), Arntz & Schmidt (1989) put this theory into perspective, since anxiety seems to intensify the pain experience only if the focus of anxiety is on the pain. This rather speaks in favor for an effect of attention and identifies anxiety as an epiphenomenon of uncontrollability, which is not necessarily causally related to the pain modulation. Another potential explanation for the effects of control is the *meaning* or *significance* of the pain in a given situation or its inferred cause (Arntz & Schmidt, 1989; Thompson, 1981). This can be illustrated in reference to Beecher's (1956) investigations of pain in soldiers. Compared to a civilian group with equal injuries, soldiers reported less pain. Beecher argued that, for the soldiers, the wounds had a positive meaning in that they had to recover at home and could escape from direct involvement in battles, whereas no positive meaning was bound to the injuries of civilians (Thompson, 1981). Further, when a pain is controllable, its meaning is changed as the pain or its cause is no longer appraised as seriously harmful or threatening (Arntz & Schmidt, 1989). One of the most accepted explanations for the effects of control on pain was posed by Miller (1979) who suggested the *minimax hypothesis*, referring to attribution theory: “A person who has control over an aversive event insures having a lower maximum danger than a person without control. This is because a person with control attributes the cause of relief to a stable internal source–his own response–whereas a person without control attributes relief to a less stable, more external source” (p. 294). This means that having control minimizes the potential maximum harm one can experience because it guarantees a stable and internal attribution of agency.

Along these lines, Wiech et al. (2006) found evidence that during self-controlled painful stimulation the right anterolateral PFC was activated, a brain area related to voluntary (i.e., conscious) (re)appraisal, at the same time the subjectively perceived intensity of the pain experience was reduced. Further imaging studies indicate that during uncontrollable compared to controllable painful stimulation,

brain areas typically associated with pain processing (primary somatosensory cortex, SI; anterior cingulate cortex, ACC; thalamus; insula; periaqueductal grey, PAG; PFC; Apkarian, Bushnell, Treede, & Zubieta, 2005) show increased activation, even if subjective ratings indicate no difference (Helmchen, Mohr, Erdmann, Binkofski, & Büchel, 2006; Mohr, Binkofski, Erdmann, Büchel, & Helmchen, 2005; Mohr, Leyendecker, & Helmchen, 2008; Salomons, Johnstone, Backonja, & Davidson, 2004). Also, subjects reporting increased pain during uncontrollable compared to controllable conditions show increased activity in pregenual ACC, PAG, and posterior insula, and secondary somatosensory cortex (SII; Salomons, Johnstone, Backonja, Shackman, & Davidson, 2007). Evidence suggests that the ACC may play a modulatory role for contextual information in the pain experience (placebo: Petrovic, Kalso, Petersson, & Ingvar, 2002; control: Salomons et al., 2004; cognitive modulation: Bantick et al., 2002; Petrovic & Ingvar, 2002). Further, the PAG is known to play a major role in descending pain modulation (Gwilym et al., 2009; Porreca, Ossipov, & Gebhart, 2002; Yoshida, Seymour, Koltzenburg, & Dolan, 2013). However, previous studies did not differentiate whether increased brain activation stemmed from augmented pain sensations or rather reflected modulatory influence.

Clinical relevance of perceived control

Controllability is important in acute (e.g., dental pain, childbirth training; Arntz & Schmidt, 1989; Thrash, Marr, & Box, 1982) as well as in chronic pain. Although it is not conclusively established which processes mediate the pain increase caused by uncontrollability or accordingly pain decrease caused by controllability, it seems unequivocal that lack of control potentially leads to learned helplessness and passivity (Müller, 2012), depression, and anxiety in the long term. Chronic pain and affective disorders often occur comorbid and lack of control is known to play a role in both disorders (Jensen & Karoly, 1991; Seligman, 1975). Chronic pain is strongly correlated with beliefs in the lack of ability to self-control pain (Philips, 1987) and generalized perceptions of no control (external locus of control, chance; Arntz & Schmidt, 1989). Learned helplessness (Abramson et al., 1978) can result in avoidance behavior which is a major factor in the maintenance of chronic pain (Samwel, Kraaimaat, Crul, & Evers, 2007; Vlaeyen & Linton, 2000). Animal research also shows that uncontrollable painful stimulation not only results in learning deficits (Mineka & Hendersen, 1985) but further in reduced food and water intake, exaggerated fear, fear conditioning, and reduced social interaction, amongst

others (Maier & Watkins, 1998) – signs reminding of symptoms in depression and anxiety disorders (Maier & Watkins, 2005).

A model explaining the relation between lack of control, pain becoming chronic, and other consequences was proposed by Arntz & Schmidt (1989). They hypothesize that chronic pain patients formerly experienced a high level of control over their physical functioning. For these patients, an acute pain event thus constitutes a major loss of control, which can be more stressful than never having had control at all (Crombez et al., 2008; Janssen et al., 2004; Staub et al., 1971). Additionally, chronic pain patients are known to oftentimes strive for total pain relief, which inevitably leads to continued failures and thus helplessness. A vicious circle between low perceived control and the experience of pain and depression potentially develops.

Therapeutic strategies usually have the goal to increase control and diminish the experience of pain, or increase perceptions of control. For example, in patient-controlled analgesia (PCA), patients in acute pain conditions (e.g., post-operative pain) get the opportunity to self-administer analgesic medication as needed and thereby control time point and amount of the drug administration. Evidence shows that due to PCA, the intake of analgesics after operations could be significantly reduced (Bennett et al., 1982). An alternative approach to deal with the issue of uncontrollability in many pain conditions is taken in the currently popular acceptance and commitment therapy (ACT; Hayes, Strosahl, & Wilson, 1999). Due to the fact that repeated, unsuccessful attempts to completely control pain (e.g., medication intake, avoidance of pain-inducing activities) can result in chronic vigilance to pain, further aggravating the condition (Crombez et al., 2008), the focus should be shifted away from the goal to control pain. The first and most important step in treating chronic pain according to ACT is to truly accept the pain before learning coping strategies (McCracken, 2004).

1.3 Methods of Pain Assessment

Pain is a multidimensional experience (Melzack & Wall, 1965) that can be assessed in different response channels, which do not necessarily have to correspond (Becker et al., 2011; Hölzl, Kleinböhl, & Huse, 2005). For example, a subject with back pain may subjectively rate the intensity of his pain as moderate, feel that it is highly aversive and that it will never get better, have a pain-specific facial

expression, and take a relieving posture or try to avoid certain movements. At the same time, this person might show increased skin conductance and muscular tension and activation in brain areas associated with pain processing. Thus pain can evoke subjective-emotional, cognitive, behavioral, and physiological reactions. In past research, pain perception has mostly been studied by means of subjective ratings (e.g., verbal and numeric pain ratings). Although they are easily applicable and possess high face validity, subjective ratings have been criticized for a number of reasons. They depend on verbal report and can consequently only assess the conscious, reportable part of the pain perception. Further, they are prone to demand characteristics ("answers of politeness and experimental subordination", p. 1314, Kienle & Kiene, 1997; Hrobjartsson & Gotzsche, 2001) and it is not clear after experimental manipulations whether the perception of the subjects or only their response criteria or categories have changed (Chapman et al., 1985). This can even happen in experimental situations without awareness for the object being evaluated (e.g., blindsight; Cowey, 2004). This suggests that subjective ratings (alone) might not be ideally suited to assess the complex experience of pain.

Pain assessment via other response channels (e.g., behavioral) is oftentimes neglected because subjective pain ratings are thought of as being sufficient. Further, behavioral responses can be more difficult to implement in an experimental procedure. A behavioral measure, i.e., discriminative behavior, is a behavioral response to a change in sensory input or subjectively experienced change in sensation (Becker, 2009; Hölzl et al., 2005). It does not depend on verbal mediation (e.g., on an instruction) and might therefore be an alternative to subjective ratings that can open up another perspective. With a behavioral measure, changes in pain perception might be observed that did not reach awareness in the subjects and would thus be missed in subjective ratings (Becker et al., 2011; Cowey, 2004). In blindsight, for instance, subjects are not aware of a visually presented stimulus and report that they did not see anything. However, in a performance test they can react accurately to the stimulus. Disadvantages of the subjective ratings, like demand characteristics, do not apply to discriminative behavior because the subjects are not necessarily aware that their responses serve as a measure of their perception. However, other than a subjective pain rating, discriminative behavior as defined above assesses *changes* in perception. This allows tracking of the dynamics of the pain perception as it develops over time. In previous studies, discriminative behavior served as a measurement for perceptual sensitization or habituation to tonic heat-pain stimulation and thereby implicitly indicating changes in pain perception. Subjective ratings, on the other side,

remained constant, suggesting that both response channels dissociated over time (Becker et al., 2011; Becker, Kleinböhl, & Hölzl, 2012; Hölzl et al., 2005; Kleinböhl et al., 1999).

Besides subjective ratings and behavioral measures, the pain experience can further be investigated by exploring its neural (e.g., electroencephalography; functional Magnetic Resonance Imaging, fMRI; magnet encephalography) and physiological correlates (e.g., heart rate, skin conductance). Although these measures cannot be equated with the pain perception itself, they can give insight into the mechanisms of pain processing, serve as correlate for the emotional dimension of pain, and help to quantify associated aspects of the pain experience, like anxiety (Chapman et al., 1985). A better understanding of the relationship between psychological and physiological factors will help in understanding pain and lead to better treatment options (Turk & Okifuji, 2002).

1.4 Aims of This Thesis

The studies presented in this thesis deal with the investigation of two pain facilitating factors, namely conditioned nocebo responses and uncontrollability, and their effect on different response channels. The influence of cognitive factors on pain perception is widely recognized, but the specific mechanisms of nocebo effects and uncontrollability remain unclear to a large extent. Further exploring those mechanisms contributes to our understanding of pain processing in healthy individuals as well as the development and maintenance of clinical pain conditions and possible ways to prevent the pain facilitating impact of both the nocebo effect and uncontrollability.

An overview over the single studies and their respective aims are given in Figure 1. In particular, the following specific aims were addressed:

Classical conditioning of a nocebo effect

In study 1, a nocebo effect in both a subjective-verbal and an implicit-behavioral response channel will be induced by classical conditioning to confirm the notion that verbal suggestions are not essential in inducing a nocebo effect in the nociceptive system.

Induction of nocebo-hyperalgesia by classical conditioning and analysis of autonomic responses and personality traits

In Study 2, the classical conditioning procedure, which was developed in Study 1, will be adapted in order to induce nocebo-hyperalgesia. Further, the effect of nocebo conditioning on heart rate and heart rate variability, as indicators of the autonomic activity, will be measured. In order to better characterize nocebo responders, motivational style and suggestibility will be assessed besides anxiety.

Exploration of the role of contingency awareness in nocebo conditioning

Classical conditioning can occur with and without contingency awareness (i.e., knowledge concerning the relation between CS and US). Although a recent study shows that a nocebo effect can be activated by subliminally presented cues after explicit conditioning (supraliminal CS presentation, Jensen et al., 2012), it is not known whether implicit conditioning (i.e., without contingency awareness) can induce a nocebo effect, which would support the notion that explicit expectations are not necessary for the nocebo effect to occur. Therefore, in both Studies 1 and 2, the role of contingency awareness and CS differentiation will be explored.

Investigation of the effect of stimulus uncontrollability on pain perception and its neural correlates

Although studies showed increased activity in pain processing brain areas during uncontrollable compared to controllable painful stimulation, it is not known which brain regions *drive* uncontrollability-induced pain augmentation. In Study 3, a yoked-control design will be developed to compare neural correlates of controllable and uncontrollable pain stimulation of the same intensity and investigate uncontrollability-induced pain sensitization during fMRI.

Methodological Aims

Methodological aims of this thesis comprise the assessment of pain-modulatory effects in both subjective-verbal and implicit-behavioral response channels. Further, a classical conditioning procedure will be developed to induce a nocebo effect. Instead of conventional artificial experimental stimuli, thermal stimuli will

serve as conditioned (CS) and unconditioned stimuli (US). Thereby we will take advantage of preparedness and natural stimulus relations, as both CS and US derived from the somatosensory domain. Another methodological aim is the development of a procedure to induce uncontrollability after the subject exerted instrumental control over the applied stimulus intensities. Here, a continuous subjective rating shall be implemented in order to uncover the pain enhancing effects and allow correlation with neural activity.

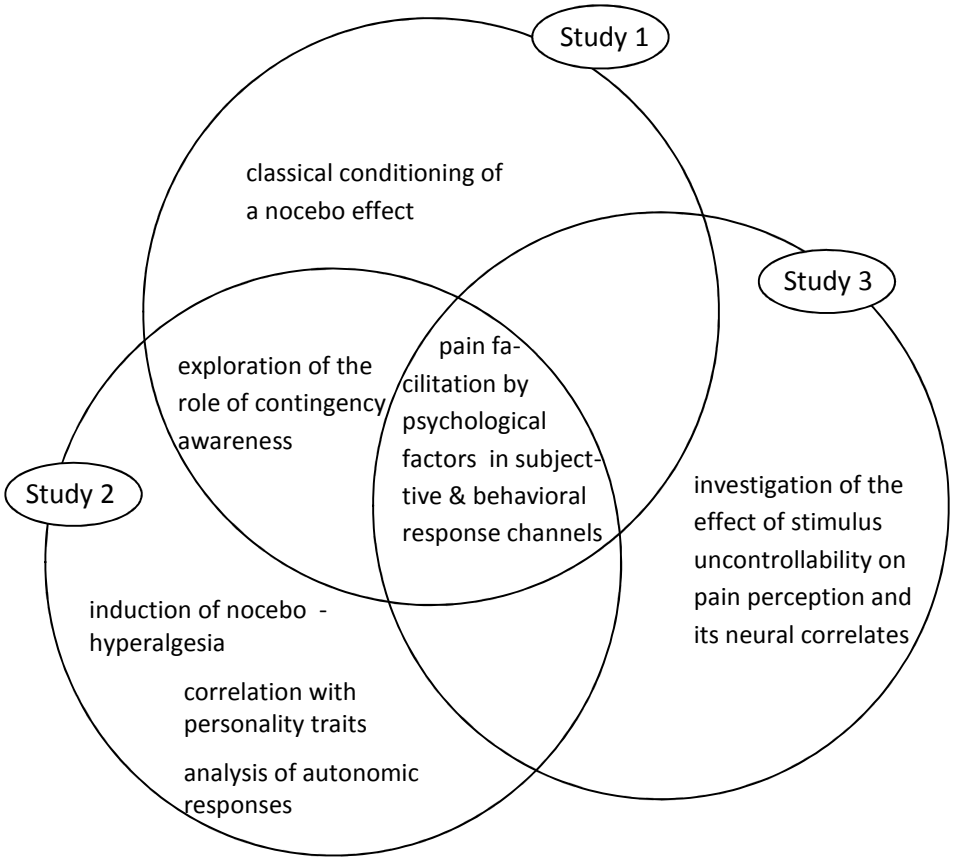

Figure 1: Overview of the studies and their respective aims. See text for details.

2. Conditioned Nocebo Effects in Heat-Pain Perception

2.1 Classical Nocebo-Conditioning of Heat-Pain Perception: Dissociation of Subjective Rating and Implicit Behavioral Response

Introduction

Placebo effects have been widely studied over the past few decades. However, their adverse counterpart, so-called nocebo effects, received far less attention (Benedetti et al., 2006). They range from feeling sick after applying an inactive substance or procedure to experiencing severe symptoms (Witthöft & Rubin, 2013), which may even lead to life-threatening conditions (Cannon, 2002). Understanding the different underlying mechanisms is highly relevant in a clinical context as nocebo effects may worsen symptoms and diminish therapy outcome (Amanzio, Corazzini, Vase, & Benedetti, 2009; Barsky, Saintfort, Rogers, & Borus, 2002; Benedetti et al., 2007; Colloca & Miller, 2011; Hahn, 1997). Nocebo mechanisms have been most widely studied in the context of pain, where they can cause hyperalgesia due to receiving an inert substance or procedure (Stewart-Williams & Podd, 2004).

As main mechanisms causing nocebo-hyperalgesia, psychological processes such as conscious expectation and classical conditioning are discussed (Enck, Benedetti, & Schedlowski, 2008; Pacheco-Lopez, Engler, Niemi, & Schedlowski, 2006; Stewart-Williams & Podd, 2004). Further, anxiety is assumed to play a role by activating the CCK-system, which in turn leads to pain increase (Benedetti et al., 2006). Evidence shows that conscious (i.e., reportable, Stewart-Williams & Podd, 2004) expectations induced by verbal instruction or explicit suggestions can result in increased reports of pain despite unchanged stimulation intensities (Benedetti et al., 2003; Colloca et al., 2008; van Laarhoven et al., 2011). However, recent results indicate that nocebo-hyperalgesia can also be learned without inducing conscious expectation (Jensen et al., 2012). Even more impressive, learned nocebo effects can be activated by masked cues, i.e., non-consciously perceived, previously neutral stimuli (Jensen et al., 2012). This learning mechanism and the non-conscious activation of nocebo effects appear to be particularly relevant in the

clinical context. For example, certain aspects of medical environments (e.g., the examination room) can serve as cues getting associated to pre-existing, ambiguous symptoms (random headache) or painful measures (taking blood samples, injections) and inadvertently trigger enhanced pain later on. Although one study in the context of placebo effects suggests that placebo-hypoalgesia could not be induced by implicit conditioning (Martin-Pichora, Mankovsky-Arnold, & Katz, 2011), it remains unclear whether a person has to be aware of the contingencies (i.e., recognize the relationship between cue and symptom) to learn and thus develop a *nocebo* effect. The significance of contingency awareness is critically debated in classical conditioning, as it seems to be necessary for successful learning in certain conditions, like delay conditioning, whereas not in others, like trace conditioning (Clark et al., 2002; Manns et al., 2001; Manns et al., 2002; Perruchet, 1985).

In most studies, nocebo-hyperalgesia has been investigated exclusively by using subjective pain reports. Although an important assessment of pain, subjective pain reports are prone to response bias (Cowey, 2004) and it is conceivable that subjective nocebo effects are caused (partly) by changes in response criteria (Hrobjartsson & Gotzsche, 2001; Kienle & Kiene, 1997). Supporting this notion, it has been shown that learning can lead to a dissociation of subjective sensation (explicit judgment of sensation) and indirectly (behaviorally) assessed perception (implicit judgment by discriminative responses; Becker et al., 2011; Hölzl et al., 2005). Similarly, nocebo effects observed in physiological correlates or secondary indicators of pain (e.g., cortisol) are not necessarily reflected in subjective measures (Johansen et al., 2003), emphasizing that pain is not a one-dimensional phenomenon. Accordingly, it is not known which behavioral consequences arise from nocebo effects although this could have important clinical implications. For example, it is known that certain behavioral responses to pain, e.g., short-term relieving and protective postures as escape and/or avoidance behavior, often unnoticed and involuntary, can lead to enhanced pain sensitivity and—in the long run—to augmented clinical pain and pain becoming chronic (Flor, Birbaumer, & Turk, 1990; Fordyce, 1976). Therefore, in order to understand the mechanisms of nocebo-hyperalgesia and its possible role in symptom worsening and chronic pain, different response channels have to be considered.

The aims of this study were to a) classically condition nocebo responses in subjectively assessed heat-pain perception, b) specify the relation of the conditioned nocebo effect to contingency awareness, c) explore short-term changes of heat-pain sensation assessed with implicit behavioral and subjective

measures (Becker et al., 2011; Hölzl et al., 2005) in response to conditioned nocebo-hyperalgesia, and d) examine the role of anxiety measures (state and trait anxiety; anxiety specifically related to pain). We hypothesized that a nocebo effect. Further, a positive correlation of the nocebo effect with anxiety measures was expected.

Methods

Subjects

Twenty-six healthy volunteers (12 females; age: M = 24.1 years, SD = 4.2 years) participated after screening for the following exclusion criteria: chronic pain (longer than three month or more than once a month for longer than three days) or acute pain, intake of analgesics or psychotropics, chronic disease (diabetes, hypertension, cardiopathy, thyroid disease, renal insufficiency, hepatic dysfunction, epilepsy, stroke, Parkinson's disease, multiple sclerosis), psychiatric or neurologic diagnoses, intake of recreational drugs, substance or alcohol abuse, pregnancy, and left-handedness (tested with the Edinburgh Handedness Inventory; Oldfield, 1971).

The experimental protocol was conducted in accordance with the revised Declaration of Helsinki and approved by the Local Ethics Committee (2010-226N-MA). All subjects gave informed consent prior to experimental testing.

Conditioning procedure

Subjects took part in one experimental session of approximately 45 min duration, during which the conditioning procedure was performed. Within each single trial, a conditioned stimulus (CS) was directly followed by an unconditioned stimulus (US; Figure 2), specifying a border case of delay conditioning (without overlap of CS and US) and trace conditioning (with a trace of 0 seconds).

The procedure was divided into a learning and a test phase. Two different thermal stimuli served as CS (see section 2.6); one served as the so-called CS– and contingently preceded the presentation of a non-painful unconditioned temperature stimulus ($US_{no\ pain}$; see section 2.5). The other served as the so-called CS+ and contingently preceded the presentation of a painful unconditioned temperature stimulus (US_{pain}; see section 2.5) in the learning phase. The two

different CS and accordingly the two different US were each presented in 15 trials in the learning phase (duration of a single trial: approximately 1 minute). In the test phase, both the CS+ and CS– preceded $US_{no\ pain}$ in five trials each, to test for conditioned nocebo responses to the CS+. Consequently, US_{pain} was not presented during the test phase. The trial order in the learning and test phases and thereby the sequence of CS+/CS– presentations was pseudo-randomized with the constraint of no more than three subsequent presentations of the same CS.

Rating scale

During the experiment, the subjects employed a visual analog scale (VAS) to indicate their sensation. The scale was vertically oriented and incorporated the sensation of temperature and pain (Kleinböhl, Trojan, Konrad, & Hölzl, 2006; Lautenbacher, Möltner, & Strain, 1992), being labeled with 0 – 'warm' at the bottom and 100 'very strong pain' at the top. At a scale value of 40, an additional anchor was included, labeled 'just painful'. The VAS was open at the upper end to avoid ceiling effects and subjects were familiarized using the VAS prior to the experimental testing.

Course of a conditioning trial

Each trial (Figure 2) started with the presentation of either the CS+ or the CS– (duration: 5 s). Following that, the stimulation intensity rose until it reached the designated temperature of either the US_{pain} or the $US_{no\ pain}$ (see section "Unconditioned Stimuli"). When the target temperature was reached, subjects rated their current subjective pain sensation on the VAS (VAS_1). Subsequently, the subjects performed a self-regulation procedure (duration: 25 s) in order to implicitly assess changes over time in subjective perception of the tonic stimulus with a behavioral response. As the CS was no longer present during this procedure, this behavioral response depicts the decomposition of the "exaggerated" subjective sensation over time and is interpreted as behaviorally assessed "decay" of the conditioned nocebo response. During the self-regulation procedure, the subjects were told to keep the temperature constant by antagonizing any perceived temperature change with a response unit (turning the wheel of a computer mouse up or down). Because the temperature did in fact not change other than when the subject operated the response unit, any change perceived by the subject was interpreted as adaption (indicated by up-regulation of the temperature) or apparent (subjective) sensitization, probably due to temporal summation (indicated by down-regulation of the temperature). After 25 s, the

temperature returned to the initial level (of US_{pain} or $US_{no\ pain}$) and the subjects rated their current subjective sensation on a VAS for a second time (VAS_2). Implementing this second subjective rating allowed us to explore an explicitly assessed indicator for change in subjective sensation (i.e., decay of the conditioned nocebo response over time, in the following called "subjective decay") besides the behavioral decay operationalized in the self-regulation procedure. After the rating, the temperature returned to baseline and 5 –10 s later the next trial started.

Unconditioned stimuli

The intensities of the US were adjusted to the subjects' individual pain thresholds that were assessed prior to the conditioning task with the method of production (Kleinböhl et al., 1999). Subjects increased the temperature themselves with the response unit, starting from baseline (34 °C) until they perceived the temperature as just painful. This assessment was repeated 3 to 6 times (taking into account inter-trial habituation processes). The just painful self-adjusted temperature of the last trial was employed as the pain threshold (Kleinböhl et al., 1999).

Two different temperatures served as US: pain threshold + 1.5 °C (US_{pain}) and pain threshold –2.2°C ($US_{no\ pain}$). The temperatures for both US were based on the pain threshold plus or minus four units of just noticeable differences (i.e., 0.37 °C above or accordingly 0.54 °C below the pain threshold; Bushnell, Duncan, Dubner, Jones, & Maixner, 1985; Maixner et al., 1986; Maixner, Dubner, Bushnell, Kenshalo, & Oliveras, 1986; Maixner, Dubner, Kenshalo, Bushnell, & Oliveras, 1989). With this approach we wanted to achieve that the two US temperatures were equally far away from the pain threshold in subjective perception although they were not equidistant physically.

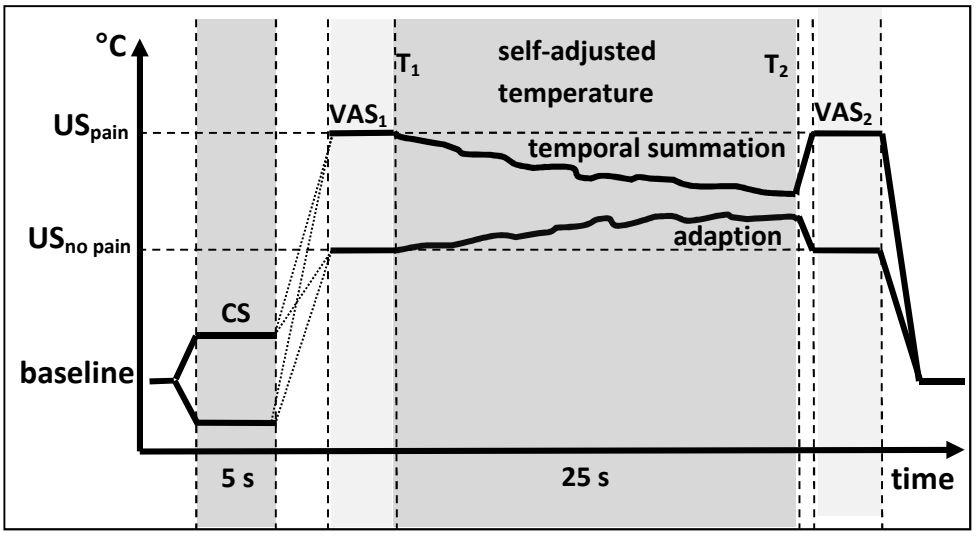

Figure 2: Trial structure. The trial started at baseline temperature (34 °C) and increased or decreased to the temperature set for the conditioned stimulus (CS; 32 °C or 36 °C). After 5 s, it increased to the preset level of the non-painful ($US_{no\ pain}$; individual pain threshold – 2.2 °C) or painful unconditioned stimulus (US_{pain}; individual pain threshold + 1.5 °C), depending on the respective assignment (dotted lines). The subject rated the currently perceived stimulus intensity on a visual analog scale (VAS_1). Then, the self-regulation procedure started (T_1, initial temperature), where the subject was told to keep the temperature constant for 25 s by operating the wheel of a computer mouse inducing cooling and heating. Depicted is an example of temporal summation after presentation of US_{pain} where the subject decreased the temperature over time and an example of adaption after presentation of $US_{no\ pain}$ where the subject increased the temperature. The difference of the initial temperature (T_1) and self-adjusted end temperature (T_2) operationalized the cumulated, implicitly assessed change in sensation ("behavioral response", interpreted as behaviorally assessed decay of the conditioned nocebo response, please refer to section "Course of a Conditioning Trial"). After the self-regulation procedure, the temperature returned to the initial intensity (T_1) and the subject rated the currently perceived stimulus intensity on a visual analog scale (VAS_2) for a second time. The difference between VAS_2 and VAS_1 operationalizes the explicitly assessed change in sensation ("subjective decay"). In the end, the temperature returned to baseline level before the next trial started.

Conditioned stimuli

Two different thermal stimuli were applied as CS; one CS had a temperature of 36 °C and the other had a temperature of 32 °C (baseline temperature +/– 2 °C). The

duration of the CS was five seconds. The coupling of the two CS with the two different US was balanced across subjects. Non-painful CS of the same modality as the painful US were used to incorporate preparedness (i.e., evolutionary advantage; Seligman, 1970) and natural relations (belongingness) instead of arbitrary coupling of CS and US (Domjan, 2005). Due to the interoceptive nature of pain (Craig, 2003), it was assumed that subjects were conditioned more likely to somatosensory (i.e., proximal body) rather than exteroceptive (i.e., distal) and artificial, experimental cues (i.e., colored squares or circles).

Apparatus for stimulus application

The experimental stimuli were applied with a contact heat thermode (25x50 mm; SENSELab-MSA Thermotest, SOMEDIC Sales AB, Sweden). The thermode system allows for phasic and tonic stimulation within a temperature range from 10 to 52 °C with a relative accuracy of 0.02 °C. The baseline temperature during the experiment was 34 °C. The rate of temperature change was 0.7 °C/s, except at the end of a trial where the temperature returned to baseline with a rate of 3 °C/s. The thermal stimuli were presented to the thenar eminence of the subject's left hand. To prevent skin damage, the maximum temperature was limited to 50 °C and total applied energy was restricted by integrating temperature over time. The procedure was terminated if a critical value was reached. This value was calculated according to human and animal data on skin burns through contact heat (Brennum, Dahl, Moiniche, & Arendt-Nielsen, 1994; Dahl, Brennum, Arendt-Nielsen, Jensen, & Kehlet, 1993; LaMotte, 1979; Pedersen, Andersen, Arendt-Nielsen, & Kehlet, 1998). The experimental procedures were automatized and controlled by a separate personal computer coupled to the thermostimulator system. A computer screen in front of the subject displayed instructions and rating scales. A computer mouse with two buttons and a mouse wheel served as response unit.

Post-experimental interview & anxiety measures

After the conditioning task, subjects were interviewed in order to assess whether they distinguished the two different CS and recognized the relationship between the CS and US, i.e.,, if they developed contingency awareness. For this purpose, subjects were shown a flowchart of the time course of one trial that had already been used during instruction. The flowchart depicted a temperature course and showed the trial sections "first temperature change" (i.e CS), "second temperature

change" (i.e., US), "first temperature rating" (i.e., VAS_1), "temperature maintenance interval" (i.e., self-regulation procedure), and "second temperature rating" (i.e., VAS_2). In order to assess successful CS+/CS– differentiation, subjects were asked if they had felt different temperatures in different trials during "first temperature change". Then they were asked whether the non-painful/painful "second temperature change" usually followed the warmer/colder stimulus and whether the colder/warmer stimulus predicted non-painful/painful "second temperature change". In case of negation, we inquired if there could have been any relation between the first and second temperature change, in particular, if one stimulus usually followed another or not.

At the end of the testing session, subjects completed both the state and trait part of the State-Trait Anxiety Inventory (STAI; Spielberger, 1970), the Fear of Pain Questionnaire (FPQ; McNeil & Rainwater, 1998) and the Pain Anxiety Symptoms Scale (PASS; McCracken, Zayfert, & Gross, 1992).

Statistical analysis

Five subjects had to be excluded from the analyses because they did not perceive the US_{pain} as painful, resulting in 21 subjects (9 females; age: M = 24.4 years, SD = 4.56 years) in the statistical analyses.

In order to test for conditioned nocebo effects, only subjective ratings during the test phase were considered (differential responses to trials cued with CS+ or CS–). However, to investigate whether potential confound variables were related to the nocebo effect and might explain differences in conditioning success, different indices derived from the learning phase (e.g., characterizing a priori differences between subjects or effects of the conditioning) were incorporated and served as additional factors or covariates, at first. Due to the significant impact of one covariate on the nocebo effect (rating of painful trials during the learning phase), the subjective ratings (VAS_1) of the test phase were adjusted accordingly by using the fitted values of the analysis of covariance for further analyses. After confirming the normal distribution of the residuals (Curran, 1996; Hair Jr, Anderson, Tatham, & Black, 1998), linear mixed models (LMM) with two fixed within-subject factors, 'CS' (CS+, CS–) and 'time' (5 trials), were used to assess the effects of the learning procedure. The intercept of 'time' served as random factor. Post hoc tests (Fisher least significant differences, LSD) were calculated to compare trials cued with CS+ and CS– across the course of the test phase where appropriate.

Indicators of subjective decay (i.e., difference between the second first subjective rating; $VAS_2 - VAS_1$) and behavioral response (i.e., difference between self-adjusted temperature in the end of each trial and initial temperature; $T_2 - T_1$) served as dependent variables within the above described LMM. Further, T-tests and Pearson's correlations were calculated where appropriate.

Post hoc, subjects were divided into subgroups of either successfully conditioned subjects (learners) or subjects who showed no conditioned responses (non-learners). Subjects were considered learners, when they, on average, evaluated the temperature following CS+ as higher compared to the temperature following CS– in the test phase (VAS_1). The learner subgroup, as allocated according to the subjective rating, was further investigated in regards to the behavioral measure, enabling an independent application of the specified learner criterion and thus avoiding circular reasoning (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009).

In order to test whether the nocebo effect was independent of contingency awareness and CS differentiation, regression analyses were calculated, in which contingency awareness and CS differentiation, respectively, predicted the size of the subjective nocebo effect (Becker et al., 2012; Dienes, 2008; Greenwald, Klinger, & Schuh, 1995). The intercept estimated the size of the subjective nocebo effect without awareness and CS differentiation, respectively, and the slope indicated whether awareness and accordingly CS differentiation were conducive to the effect.

The significance level was set to 5%. Figures were prepared in R 2.8 (R Development Core Team, 2010) and statistical tests were calculated in SPSS 21.

Results

Manipulation check

The mean pain threshold of the subjects was 43.4 °C (SD = 2.64), resulting in average stimulus temperatures of 41.2 °C for the non-painful and 44.9 °C for the painful US. Subjects clearly distinguished between the painful (M = 60.8, SD = 15.72) and non-painful US (M = 8.5, SD = 9.17) in the subjective rating during the learning phase (VAS_1; main effect 'CS': $F(1, 64.3) = 1020$, $p < .001$). The ratings increased over time (Figure 3A; main effect 'time': $F(14, 194.9) = 2.52$, $p = .002$),

which was more the case for the painful trials (interaction effect 'CS x 'time': F(14, 242.3) = 7.01, p < .001), indicating sensitization across trials.

Possible confound variables related to the nocebo effect

We investigated whether the nocebo effect in the subjective ratings was influenced by a number of confounding variables. The following variables did not impact the nocebo effect in the subjective ratings:

a) *sensitization across trials during the learning phase* (distinguishing sensitizers (beta ≥ .1; N = 10) from non-sensitizers (beta > .1; N = 11) by predicting the subjective ratings of the painful stimulation over time during the learning phase in regression analyses for each subject; main effect 'sensitization': F(1, 20.8) = .74, p = .399; interaction effect 'CS' x 'sensitization': F(1, 39.7) = .79, p = .379);
b) *distribution of subjective ratings of the painful trials during the learning phase* (classifying subjects according to their deviation from the Gaussian distribution, for more details please refer to Figure SI A in the Appendix; main effect 'deviation': F(1, 20.9) = 1.03, p = .322; interaction effect 'CS' x 'deviation': F(1, 39.4) = 1.05, p = .313);
c) *pain threshold* (main effect 'pain threshold': F(1, 91.4) = 18.4, p < .001; interaction effect 'CS' x 'pain threshold': F(1, 128.8) = 0.46, p = .497);
d) *average difference in adaptation between non-painful and painful trials on an individual basis during the learning phase* (main effect 'adaptation': F(1, 94.4) = 0.27, p =.606; interaction effect 'CS' x 'adaptation': F(1, 125.5) = 4.87, p = .029);
e) *difference in subjective ratings between non-painful and painful trials during the learning phase* (main effect 'VAS difference': F(1, 94.8) = 2.79, p =.098); interaction effect 'CS' x 'VAS difference': (F(1, 124.6) = 13.92, p < .001);
f) *degree of temporal summation or adaption in response to painful trials (CS+) during the learning phase* (main effect 'adaptation CS+': (F(1, 94.2) = 2.04, p =.157; interaction effect 'CS' x ' 'adaptation CS+': F(1, 127.2) = 0.911, p = .342).

Finally, including the *subjective rating of the painful trials during the learning phase* led to a main effect (main effect 'US rating': F(1, 88.6) = 51.6, p < .001) as well as an interaction effect (interaction effect 'CS' x 'US rating': F(1, 125.5) = 30.1,

p < .001). In order to account for this variance induced by the perception of painful trials in the learning phase, we used the fitted values of subjective ratings for the following analyses.

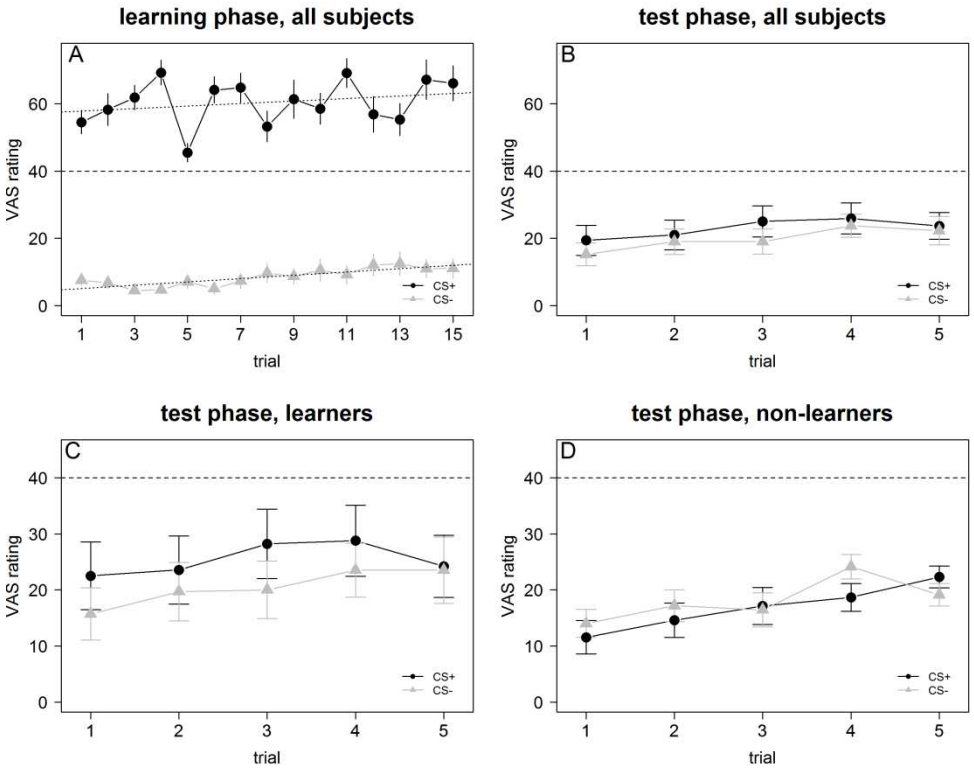

Figure 3: Subjective ratings (VAS_1) throughout the experiment. Depicted are subjective ratings (mean and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and the test phase (fitted values, adjusted for differences in the rating of the painful stimuli during the learning phase; B) of the whole sample (N = 21) and during the test phase of the learner subgroup (C; N = 13), and non-learner subgroup (D; N = 8). The dashed line depicts the pain threshold (VAS = 40).

Nocebo effects in subjective ratings

A nocebo effect in the subjective ratings (fitted values, see section "Statistical Analysis") was observable in the test phase: the same stimulation intensity was rated higher when cued by the CS+ (initially coupled to US_{pain}) compared to the CS–

(initially coupled to $US_{no\ pain}$; main effect 'CS': $F(1, 39.8) = 5.37$, $p = .026$; Figure 3B). Further, subjective responses increased over time (main effect 'time': $F(4, 86.5) = 4.64$, $p = .002$), but this was not different for the two CS (interaction effect 'CS' x 'time': $F(1, 95.1) = 1.04$, $p = .393$).

Fifteen out of 21 subjects (71.4 %) were successfully conditioned as identified by larger ratings of the temperature (VAS_1) when cued by CS+ compared to CS– (Figure 3C; please also refer to the non-learners in Figure 3D). Subjective ratings did not change across trials during the test phase (main effect 'time': $F(1, 82.7) = 1.18$, $p < .328$). An interaction effect (Figure 3C; interaction effect 'CS' x 'time': $F(4,80.8) = 4.63$, $p = .002$; main effect 'CS': $F(1, 80.8) = 67.6$, $p < .001$) indicated that extinction occurred, which was confirmed by post hoc tests (trial 1: LSD = 6.8, $p < .001$; trial 2: LSD = 3.9, $p = .005$; trial 3: LSD = 8.2, $p < .001$; trial 4: LSD = 5.3, $p < .001$; trial 5: LSD = 0.7, $p = .621$).

Subjective decay of the nocebo response

For an illustration of the subjective ratings after the self-regulation procedure (VAS_2), please refer to Figure SII in the Appendix.

To explore how the subjective sensation developed over time within trials, the difference between VAS_2 and VAS_1 was calculated. In the learning phase, negative values for trials cued with CS+ indicated that the second subjective rating was smaller than the first subjective rating within one trial, whereas no difference between first and second rating was observable for trials cued with CS– (Figure 4A; main effect 'CS': $F(1, 120.5) = 8.9$, $p = .003$). The difference in the ratings changed across the learning phase (Figure 4A; main effect 'time': $F(14, 220.1) = 3$, $p < .001$). Further the interaction effect (interaction effect 'CS' x 'time': $F(14, 266.7) = 4.48$, $p < .001$), which depended on increasingly negative differences between VAS_2 and VAS_1 within CS+ but not CS– trials, indicated increasing subjective decay of the conditioned nocebo response over time (i.e., across the trials).

In the test phase, the difference in subjective ratings indicated increased subjective decay for trials cued with CS+ compared to CS–, as well (Figure 4B; main effect 'CS': $F(1, 141.1) = 12.67$, $p = .001$). There was neither a change across trials (main effect 'time': $F(4, 169.4) = 0.6$, $p = .663$) nor an interaction effect (interaction effect 'CS' x 'time': $F(4, 141.1) = 0.2$, $p = .938$), suggesting that this difference depending on CS type was stable. This pattern of results was confirmed when only testing the learner subgroup (Figure 4C; main effect 'CS': $F(1, 99.3) = 11.76$, $p =$

.001; main effect 'time': F(4, 117.2) = 0.41, p = .802; interaction effect 'CS' x 'time': F(4, 99.3) = 0.26, p = .905).

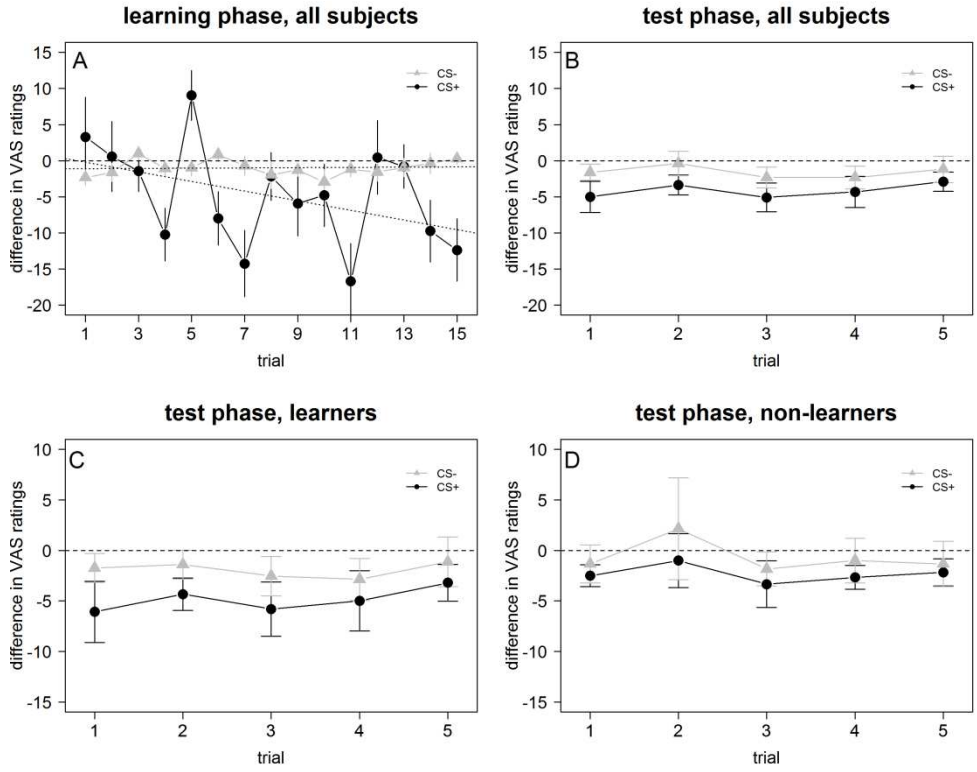

Figure 4: Subjective decay of the conditioned nocebo effect ($VAS_2 – VAS_1$) throughout the experiment. Depicted are the differences in subjective ratings (means and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and test phase (B) of the whole sample (N = 22) and during the test phase of the learner subgroup (C; N = 11) and non-learner subgroup (D; N = 11).

Self-regulation procedure

In the test phase, for the whole sample, no difference in response to trials cued with CS+ and CS– was apparent in the behavioral response (main effect 'CS': *F*(1, 131.5) = 2.07, *p* = .153) and there was no change over time (main effect 'time': *F*(4, 176.2) = 1.17, *p* = .324; interaction effect 'CS' x 'time': *F*(4, 131.5) = 0.08, *p* = .988).

For trials of both CS type, subjects' behavioral responses indicated adaption to the stimulation (positive values; Figure 5B).

When restricting the analysis to the learner subgroup, based on subjective ratings, analyses showed that they adapted more within trials when cued with CS+ compared to CS–, indicating a stronger decay of the conditioning effect during the trial (Figure 5C; main effect 'CS': $F(1, 93.9) = 4.2$, $p = .043$). No main effect of 'time' ($F(4, 125.6) = 0.67$, $p = .615$) and no interaction effect emerged (interaction effect 'CS' x 'time': $F(4, 93.9) = 0.04$, $p = .997$), suggesting that the conditioned effect remained stable over time.

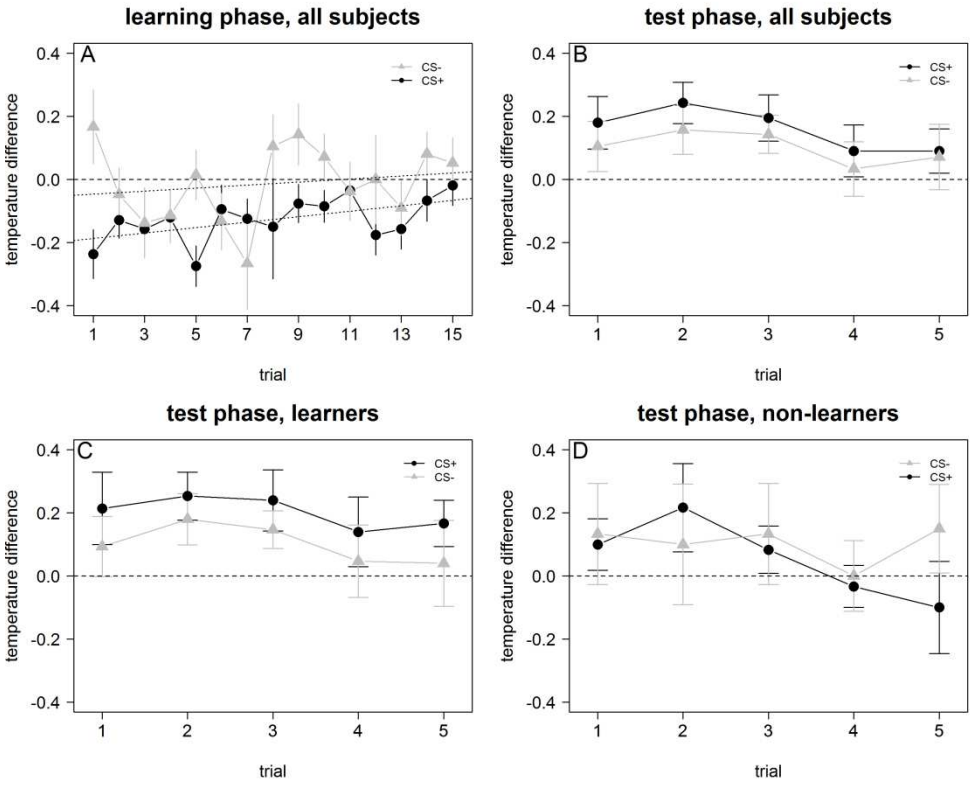

Figure 5: Behavioral response (i.e., behaviorally assessed decay of the conditioned nocebo response) throughout the experiment. The y-axis represents the difference between self-adjusted end temperature and initial temperature of the subjective adaption procedure (mean and standard errors of mean) of the single trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and test phase of the whole sample (B; N = 21) and the test phase of the learner subgroup (C; N = 13) and non-learner subgroup (D; N = 8).

In the learning phase, subjects showed more temporal summation in response to the painful trials as compared to non-painful trials (Figure 5A; main effect 'CS': $F(1, 447.7) = 16.7$, $p < .001$). There was no main effect over time (main effect 'time': $F(14, 524.7) = 1.3$, $p = .202;9$) but the interaction indicated that over time (across trials) the degree of temporal summation decreased for the painful trials (interaction effect 'CS' x 'time': $F(14, 440.4) = 1.8$, $p = .036$).

Contingency awareness and differentiation of CS temperatures

Remarkably, fourteen out of 21 subjects (66.7 %) did not recognize the contingency between CS and US, i.e., could not tell if 32 or 36 °C was coupled to the higher US temperature. None of the subjects in the non-learner subgroup was aware of the contingency; however, only seven subjects out of the learner subgroup (46.7 %) were contingency aware, indicating that contingency awareness was not necessary for successful conditioning (effect size of contingency awareness: d = 0.27). The positive intercept in the regression analyses confirmed the notion that learning of a nocebo response was independent of contingency awareness. (Figure 7A; intercept = 7.6) Further, the slope (slope = -0.03) revealed that contingency awareness was neither helpful nor detrimental.

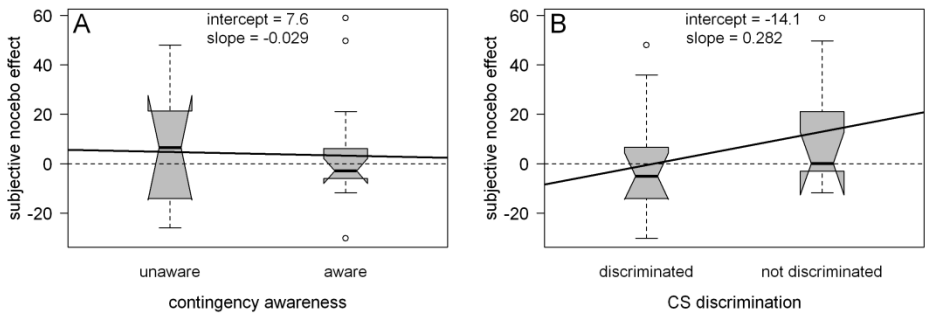

Figure 7: Regression of nocebo effect in the subjective rating with contingency awareness (A) and differentiation of the conditioned stimuli (CS; B). Subjects were categorized as aware or unaware of the contingency between CS and US and either distinguished or could not distinguish between CS+ and CS−. The notch of the box plots displays the median and the length of the notched bar displays a confidence interval around the median.

Nine subjects out of 21 (42.9 %) stated in the interview after the experiment that they could not distinguish between CS− and CS+ (32 and 36 °C). Three subjects of the non-learner subgroup (60 %) and six subjects of the learner subgroup (40 %) could not distinguish the CS, indicating that conscious differentiation of the two CS was neither necessary nor sufficient for successful conditioning. However, the negative intercept in the regression analyses indicated that the subjective nocebo effect depended of CS differentiation (Figure 7B; intercept = -14.1) and the positive slopes further revealed that differentiation of the CS would have increased learning (slope = 0.28).

Anxiety measures

The subjective nocebo effect was not associated with state ($r = -0.35$, $p = .116$) or trait anxiety (STAI; $r = -0.07$, $p = .766$), fear of pain (FPQ; all $p > .344$) or fear and anxiety responses specific to pain (PASS; all $p > .067$), suggesting that differences in anxiety were not related to the learning success.

Discussion

The present results demonstrate successful learning of a nocebo effect, i.e., increased perception of nociceptive stimuli indicated by subjective ratings in a respondent learning procedure. In the absence of the nocebo cue, a decay of the nocebo response was observable in both the subjective and the behavioral responses (the latter only in the learner subgroup), indicated by increased adaption within the trial. The majority of subjects did not recognize a relation between the cues and the following stimulation and contingency awareness was not necessary for the nocebo effect to occur. However differentiation of the CS was beneficial. Contrary to our hypothesis, anxiety was not related to the subjective nocebo effect.

To our knowledge, this is the second study to induce a conditioned nocebo effect without additional verbal suggestions or nocebo cues that are prone to induce expectations from the outset (Jensen et al., 2012). This demonstrates that conditioning is sufficient to generate a nocebo effect and conflicts with the hypothesis that learning is unimportant in nocebo-hyperalgesia, compared to placebo-hypoalgesia (Colloca et al., 2008). Further, for the first time, the short time development of sensation after having triggered a nocebo response was

investigated. In contrast to simultaneous or clear-cut trace conditioning, in which the CS would co-terminate with the US, the present conditioning paradigm, which was a borderline case of delay conditioning (without overlap of CS and US) and trace conditioning (duration of the trace between CS and US = 0) enabled us to observe the development of the conditioned response over time because the CS was directly followed by the US and did not overlap. Figuring that the conditioned nocebo response should decompose over a time range of a few seconds if the nocebo cue is no longer present, the increased adaption in subjective sensation was interpreted as decay of the nocebo response. In other words, the results suggest that the "exaggerated subjective perception" induced by the nocebo-conditioning was reset shortly after it had been provoked.

From this point of view, evidence for an increasing conditioning effect during the learning phase might be assumed: The sensitization across trials in VAS_1 (i.e., increasing subjectively reported intensity) might be interpreted as partially caused by the conditioning in the face of the simultaneously increasing subjective decay, representing the nocebo portion of this sensitization.

The decay of the nocebo response was observable in both the subjective and behavioral measure. Forming an explicit judgment and producing a communicable response requires many cognitive operations, which makes the process of subjective rating interference-prone for external influences, such as demand characteristics (Hrobjartsson & Gotzsche, 2001; Kienle & Kiene, 1997), judgment bias, and changes in response criteria (Cowey, 2004). However, the implicit behavioral measure does not depend on verbal mediation and thereby reduces the risk to confound changes in response criteria with changes in perception (Cowey, 2004). The results indicate that the subjective nocebo effect was not solely caused by demand characteristics (Hrobjartsson & Gotzsche, 2001; Kienle & Kiene, 1997) as a nocebo effect was also observed in the behavioral measure; and during the self-regulation procedure subjects were not necessarily aware that their sensation was assessed (Hölzl et al., 2005). In addition to that, with the behavioral measure aspects of perception can be assessed that are not represented verbally (e.g., Becker, 2009; Gazzaniga, 2005; Weiskrantz, 2004). Accordingly, the results show that the subjective-verbal and implicit-behavioral response channels cover partly different aspects of perception, since the decay of the nocebo response, as assessed with the self-regulation procedure, was only apparent in the learner subgroup. This has important clinical implications because evidence shows that behavioral responses to changes in pain sensation can occur unnoticed by the patient (Becker et al., 2011; Hölzl et al., 2005) and contribute to increased pain

sensitivity in the long run. The investigated sample consisted of healthy subjects that showed a quick decay of the nocebo response, which could be interpreted as a resilience factor. However, it is conceivable that the decay of the nocebo response is deficient in patients with acute or chronic pain (e.g., delayed/decreased decay or prolonged nocebo response in subjective or behavioral assessment) and thereby further exacerbating pain sensitivity or promote adverse behavior (e.g., relieving postures).

There are possible alternative explanations other than decay of the nocebo response for the observed pattern in the change of the subjective perception, which cannot be ruled out, yet not necessarily contradict our interpretation. The increased adaption in subjective perception following a nocebo response could depend on contrast effects (Gibson, 1937). As the size of the contrast effect depends on the initial value, it would be larger after subjectively increased pain perception (i.e., after CS+, when a subjective nocebo effect was induced) and consequently lead to a stronger decrease in perception compared to trials cued with CS–. Further, the decreased perception could be due to descending inhibition, an opposite process that might build up parallel to the nocebo induction, yet proceed more slowly. If this was true, our experimental design might be able to identify subjects with deficient descending inhibition that are thus susceptible to nocebo effects. Evidence shows that expectations of hyperalgesia blocked the analgesic effects of descending inhibition on spinal nociceptive reflexes (RIII-reflex) and perceived pain in a DNICS design (Goffaux et al., 2007); however it is possible that the mechanisms are different for nocebo induction by conditioning. Accordingly, the CS could serve as trigger for the activation of the descending control system.

Whereas one previous study indicated that contingency awareness is a necessary condition for placebo-hypoalgesia (Martin-Pichora et al., 2011), the present results show that this is not the case for nocebo effects. Similarly, Jensen et al. (2012) demonstrated that non-consciously perceived cues can activate nocebo-hyperalgesia after explicit conditioning, although contingency awareness was not assessed. Without contingency awareness explicit expectations cannot develop during conditioning, further supporting the notion that conditioning without explicit expectations is sufficient to induce a nocebo effect. In general, whether awareness is a necessary condition for successful classical conditioning is still a question of debate (Clark et al., 2002; Lovibond, 2002). Evidence shows that one determining factor might be the design of the conditioning procedure. For successful eyeblink conditioning, awareness seems to be required for trace

conditioning (i.e., the CS is terminated before the US starts), but awareness does not seem to be necessary and rather an epiphenomenon, at most, in delay conditioning (i.e., presentation of CS and US overlap and co-terminate; Clark et al., 2002; Manns et al., 2001; Manns et al., 2002; Perruchet, 1985). As already mentioned, the procedure used in this study represents a borderline case in terms of delay or trace conditioning. Further, other than in past nocebo studies (Colloca & Benedetti, 2006; Colloca et al., 2009; Colloca et al., 2010; Jensen et al., 2012), temperature stimuli rather than visual cues were employed as CS. By this means, CS and US were both thermal stimuli and processed by overlapping systems. We assumed a better comparability to clinical settings due to preparedness (i.e., evolutionary advantage; Domjan, 2005; Seligman, 1970) and because it seemed more likely that patients are conditioned to interoceptive rather than artificial experimental cues (i.e., colored squares or circles), as pain can be viewed as a homeostatic emotion, comparable to other homeostatic modalities including temperature (Craig, 2003). It is thus conceivable that the choice of stimuli promoted implicit conditioning. Accordingly, some subjects developed a subjective nocebo effect even though they were not contingency aware and contingency awareness was not helpful for the subjective nocebo effect. In a clinical context, this means that symptoms can become unconsciously conditioned to (random) cues that in turn can, unbeknownst to the patient, trigger these symptoms later. This might lead to distrust in therapeutic efficacy, aggravation of illness, and unnoticeably contribute to the maintenance of chronic pain (Flor et al., 1990; Flor, 2000).

Not surprisingly, a conditioned nocebo effect could not be induced in all subjects. Whereas approximately one third of the subjects usually show a placebo response in according studies (Beecher, 1955; Hoffman, Harrington, & Fields, 2005), it is not known, so far, whether the same responder rate applies to the nocebo effect. In the presented study, 71.4 % of the subjects were considered nocebo responders when using a criterion that was based on the differential response to both CS in the subjective ratings. Applying this criterion resulted in a greater number of responders compared to studies in which a median split was conducted to divide the sample into “high and low responders” (e.g., Elsenbruch et al., 2012; Scott et al., 2007; Scott et al., 2008), but at the same time decreased the effect size of the nocebo effect as subjects with small nocebo responses were considered responders, too. However, to avoid capitalization of chance (Kriegeskorte et al., 2009), the described criterion that differentiated between learners and non-learners was applied only

to analyze the behavioral assessment of the nocebo decay.

Only few studies were concerned with the question whether nocebo responders possess specific characteristics (Barsky et al., 2002; Drici, Raybaud, De Lunardo, Iacono, & Gustovic, 1995). Other than in a previous study (Colloca et al., 2010), state and trait anxiety were not associated with the subjective nocebo effect. The nocebo response also did not correlate with pain-related measures of anxiety. Our results rather suggest a relation between size of the subjective nocebo response and pain sensitivity (i.e., subjective rating of the painful US in the learning phase), but not pain threshold and thus physical stimulus intensities. Whether this relation between heat-pain sensitivity and nocebo response is mediated by activation of the descending inhibitory system remains to be determined.

In summary, this study indicates that nocebo effects in heat-pain perception can be induced by learning alone and that contingency awareness is not necessary. Observing the development of the conditioned response over time, its decay was apparent in both the subjective and behavioral measure, indicated by increased adaption. The nocebo response was not related to anxiety assessed with questionnaires but covaried with pain sensitivity, which might be due to differences in descending control. The results have important clinical implications because they suggest that nocebo effects can be (unnoticeably) learned and cause adverse effects. Future studies should investigate whether the mechanisms (e.g., decay of the nocebo response in the behavioral measure) are altered in patients with chronic pain conditions.

2.2 Conditioned Nocebo-Hyperalgesia and Its Relation to Autonomic Indices and Personality Traits

Introduction

The theoretical background for Studies 1 and 2 is largely identical. Study 2 is an advancement of Study 1 in methodological and technical terms. Due to the overlap in the theoretical background, only changes in Study 2 compared to Study 1 are introduced here.

One aim of this study was to induce nocebo-hyperalgesia, i.e., a nocebo effect within the subjectively painful range. In Study 1, non-painful and moderately painful stimuli were employed as unconditioned stimuli during learning and only the non-painful stimulus was presented during the test phase. Due to the features of the VAS, which was dissected in areas describing painful as well as non-painful sensations (Lautenbacher et al., 1992), we were able to measure a conditioning-induced nocebo effect in the non-painful range. In this study, however, we increased the stimulus intensities used throughout the experiment and employed a mildly painful and a moderately painful stimulus in the learning phase and accordingly presented only the mildly painful stimulus during the test phase. Further, we based the determination of the presented stimulus temperatures no longer on the pain threshold but used a method that depended on the subjective perception of the stimuli (Montgomery & Kirsch, 1997; Price et al., 1999; Voudouris et al., 1985). It seems more promising to use subjective perception instead of a psychophysical entity (i.e., the pain threshold) as a basis for the determination of the stimulus intensities for reaching the goal of inducing a change in subjective perception.

A second purpose of this study was to explore effects of the nocebo conditioning procedure on the autonomic system. Investigations of psychophysiological responses on the nocebo effects are rare, but findings on this regard might increase insight into underlying mechanisms of the nocebo effect. For example, according to Benedetti (Benedetti, Amanzio, Casadio, Oliaro, & Maggi, 1997; Benedetti & Amanzio, 1997; Benedetti et al., 2006; Colloca & Benedetti, 2007) nocebo-hyperalgesia is mediated by anxiety-triggered CCK-activation and could be blocked with benzodiazepines that abolished HSA-hyperactivity as well as hyperalgesia. Accordingly, one study found increased cortisol levels after nocebo

suggestions, supporting involvement of stress and anxiety (Johansen et al., 2003). Further, another study observed reduced increases in heart rate and sympathetic responses during tonic ischemic noxious stimulation after inducing a placebo effect by saline versus naloxone injection (the latter reversing the placebo effect dependent on opioid systems; Pollo et al., 2003). Atropine injections, however left the placebo-hypoalgesia and heart rate responses unaffected, indicating that the parasympathetic system was not involved (Pollo et al., 2003). Heart rate measures the net effect of autonomic heart control by the sympathetic and parasympathetic nervous system in addition to humoral control. Heart rate variability (HRV) on the other hand, is a function of cardiorespiratory modulation and indicates parasympathetic heart control that is mediated only by the vagus nerve. Measures of the time-domain can be employed already for analyzing short periods of time (e.g., the duration of one trial as implemented in this study), whereas frequency-domain measures rely on longer time periods. We hypothesized an increase in heart rate and a decrease in HRV after induction of nocebo-hyperalgesia due to results indicating reduced parasympathetic activation in response to stress and anxiety (Dishman et al., 2000).

Whereas a number of different personality traits seem to correlate with susceptibility for the placebo effect (e.g., hypnotizability/suggestibility: Wickramasekera, 1980; De Pascalis, Chiaradia, & Carotenuto, 2002; dispositional optimism: Morton, Watson, El-Deredy, & Jones, 2009; reward-related personality traits: Schweinhardt, Seminowicz, Jaeger, Duncan, & Bushnell, 2009), not much research has investigated personality traits correlating with the nocebo response (apart from adverse side effects in drug trials; e.g., Andrykowski & Redd, 1987; Uhlenhuth et al., 1998; Geers, Helfer, Kosbab, Weiland, & Landry, 2005; Papakostas et al., 2004; Uhlenhuth et al., 1998). So far, only anxiety has been shown to correlate with the nocebo effect (Bingel et al., 2011; Colloca et al., 2010). However, Study 1 did not reveal a correlation of the nocebo response with state or trait anxiety or anxiety measures related to pain, as a number of other studies, too (Schmid et al., 2013; Vogtle et al., 2013). Due to the results from placebo research, in addition to anxiety, we therefore assessed suggestibility and motivational style in the subjects to explore whether a relation of these traits to the nocebo response exists.

Thus, the aim of this study was fourfold: (1) Besides replicating results of Study 1 (i.e., subjective nocebo response; decay of the nocebo response as indicated in subjective and behavioral measures), we strived to (2) induce nocebo-hyperalgesia, (3) investigate autonomic indices of the nocebo response, and (4)

explore the relations of anxiety, suggestibility, and motivational style with the nocebo response.

Methods

The methods employed in Study 2 were identical to Study 1 (please refer to section 2.1), except for the following details.

Subjects

Twenty-two healthy volunteers (12 females; age: M = 22.6 years, SD = 3.34) participated in the study.

Conditioning procedure

Every subject performed one session of about 55 minutes duration. A trace conditioning design was implemented in this study (please refer to section Conditioned stimuli). The test phase of the conditioning task was prolonged in that each of the two CS–US combinations was presented in seven instead of five trials and further amended by three booster trials, i.e., the presentation of CS+ followed by $US_{moderate\ pain}$ (cf. section Unconditioned Stimuli). The booster trials were implemented in order to enhance the learning effect and prevent extinction. The trial order and thereby the sequence of CS+/CS– presentations was randomized with the constraint of no more than three subsequent presentations of the same CS.

Unconditioned stimuli

To determine the intensities of the US, 24 temperature stimuli between 38 and 48.5 °C were pseudorandomly applied for five seconds each, with an inter-stimulus-interval of 10 s. One second after the target temperature was reached, the subject rated the perceived intensity on a VAS. Individual temperatures corresponding to a rating of 50 and 70 were estimated by robust regressions after completion of all stimuli and served as $US_{mild\ pain}$ and $US_{moderate\ pain}$, respectively. In contrast to Study 1, we chose this approach (Price et al., 1999) to determine the US intensities because we sought to apply an equally painful level of stimulation in

subjective perception in all subjects and wanted to warrant that the stimuli were in the subjectively painful range.

Conditioned stimuli

To increase differentiation of the CS, a short temperature pulse was employed as CS rather than the presentation of a constant temperature of five seconds duration because dynamically changing stimuli are easier to detect than static stimuli (Stančák, Mlynář, Poláček, & Vrána, 2006). Thus, starting at baseline (34 °C), the temperature changed to 32 °C or accordingly 36 °C and after one second shortly returned to baseline before the presentation of the US.

Heart rate assessment

To measure heart rate, the subjects wore a POLAR heart rate monitor (RS800) and a transmitter with ECG electrodes attached to the chest during the conditioning procedure. The equipment recorded the heartbeat peak-to-peak (in ms). Artifacts were corrected with the program Polar Pro Trainer. The data was exported to R and analyzed with the R package R-HRV (Rodriguez-Linares, Vila, Mendez, Lado, & Olivieri, 2008). To reconstruct the time axis, the heart rate was interpolated to attain a periodic time frame (4 Hz). After low pass-filtering, average heart rate (indicating sympathetic, parasympathetic, and humoral heart control) and HRV parameters (indicating parasympathetic heart control) for each trial were calculated. Due to equipment failure and errors in data acquisition, physiological data of four subjects had to be discarded.

Questionnaires

In order to assess motivational style, the behavioral inhibition system and behavioral activation system scales (BIS/BAS; Carver & White, 1994; in German: Strobel, Beauducel, Debener, & Brocke, 2001) was assessed. Further the subjects filled out the Tellegen Absorption Scale (Tellegen & Atkinson, 1974; in German: Ritz & Dahme, 1995) measuring absorption, a disposition positively correlated with hypnotizability and suggestibility.

Results

Manipulation check

In the learning phase of the experiment, the subjects clearly distinguished between the mildly (M = 29.3, SD = 19.47) and moderately painful US (M = 64.4, SD = 25.88) in the subjective ratings (main effect 'CS': F(1, 474) = 1080.2, p < .001) and sensitized over time (Figure 8A; main effect 'time': F(14, 514) = 6.07, p < .001), which was more pronounced for moderately painful trials (cued with CS+; interaction effect 'US' x 'time': $F(14, 473.9) = 2.76$, $p = .001$).

Possible confound variables related to the nocebo effect

In order to investigate possible confounding variables covering a nocebo effect we systematically explored a number of potential candidate variables, as in Study 1. However, none of the following variables showed a relation to the subjective nocebo effect:

a) *sensitization in response to the moderately painful trials during the learning phase* (please refer to Study 1; main effect 'sensitization': F(1, 122.6) = 5.73, p = .018; interaction effect 'CS' x 'sensitization': F(1, 196.6) = .55, p = .457);
b) *distribution of subjective ratings of the painful trials during the learning phase (classifying* subjects according to their deviation from the Gaussian distribution; for more details please refer to Figure SI B in the Appendix; main effect 'deviation': (F(2, 92.4) = 71.6, p < .001; interaction effect 'CS' x 'deviation': F(2, 209.4) = 0.05, p = .955);
c) *US temperatures* (main effect 'temperature': F(1, 110.3) = 5.26, p = .024; interaction effect 'CS' x 'temperature': F(1, 177.2) = 1.73, p = .19);
d) *average subjective ratings of moderately painful trials during the learning phase* (main effect 'US rating': F(1, 89.5) = 173, p < .001; interaction effect 'CS' x 'US rating': F(1, 216.5) = 0.11, p = .737);
e) *difference in the subjective ratings between mildly and moderately painful stimulation in the learning phase* (main effect 'VAS difference': (F(1, 124.9) = 11.5, p = .001; interaction effect 'VAS difference': F(1, 200.9) = 0.42, p = .518);
f) *average difference in temporal summation or adaption between moderately and mildly painful trials* (main effect 'adaption': (F(1, 120.1) =

12.7, p = .001; interaction effect 'CS' x ' 'adaption': F(1, 195.4) = 0.29, p = .59);

g) *degree of temporal summation or adaption in response to moderately painful trials during the learning phase* (main effect 'adaption CS+': (F(1, 118.3) = 25.2, p < .001; interaction effect 'CS' x ' 'adaption CS+': F(1, 197.7) = 0.18, p = .676).

Subjective nocebo effects

When comparing the subjective ratings of the mildly painful US cued by the CS+ and the CS–, no nocebo effect was observable (main effect 'CS': F(1, 195) = 1.14, p = .29; Figure 8B), meaning that the subjects did not rate trials cued with CS+ or CS– differently.

Eleven out of 22 subjects (50 %) were successfully conditioned as indicated by higher intensity ratings of the temperature when cued by CS+ compared to CS–. When the analysis of the nocebo effect was restricted to this learner subgroup, a nocebo response could be demonstrated, as expected (main effect 'CS': F(1, 95) = 14.58, p < .001). The learners rated the US as clearly painful (M=52.0, SD=26.2), while non-learners perceived the $US_{mild\ pain}$ as just about painful on average (M = 40.5, SD = 22.3; T = 5.93, p < .001; Figure 8D).The subjective ratings did not change across trials in the test phase (Figure 8C; main effect 'time': $F(6, 102) = 0.86$, $p = .53$) and no interaction effect appeared, indicating that the conditioned effect remained stable across the test phase and no extinction occurred (interaction effect 'CS' x 'time': $F(6, 95) = 1.88$, $p = .091$).

Post hoc analyses revealed that the learner group received higher US temperatures ($US_{moderate\ pain}$: M = 46.2°C, SD = 1.15; $US_{mild\ pain}$: $M_{learners}$ = 44.6°C, SD = 0.95) compared to the non-learners ($US_{moderate\ pain}$: M= 45.2 °C, SD = 1.05; T = 2.22, p = .038; effect size: d = 0.91; $US_{mild\ pain}$: M = 43.6 °C, SD = 1.05; T = 2.15, p = .044; effect size: d = 1). However, the subjective ratings of $US_{moderate\ pain}$ and $US_{mild\ pain}$ were not different between learners ($US_{moderate\ pain}$: M = 65.2, SD = 26.01; $US_{mild\ pain}$: M = 31, SD = 22.38) and non-learners during the learning phase ($US_{moderate\ pain}$: M = 63.5, SD = 25.75; T = 0.59, n.s.; $US_{mild\ pain}$: M = 27.6, SD = 15.85; T = 1.61, n.s.), indicating that the stimuli were perceived similar on a subjective level.

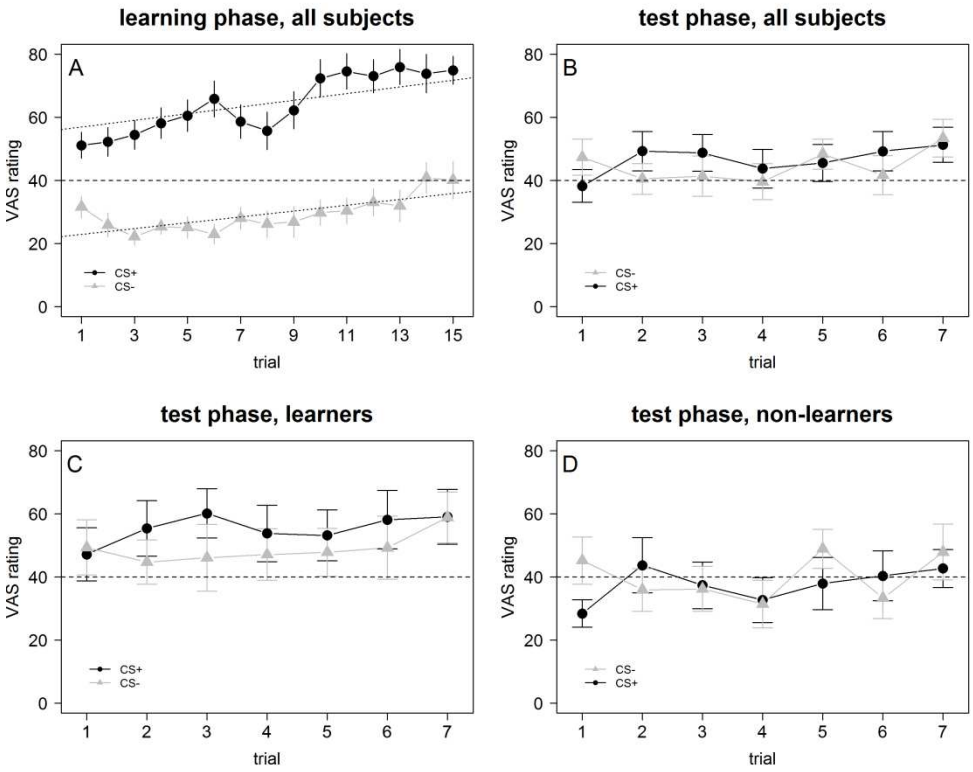

Figure 8: Subjective ratings (VAS_1) throughout the experiment. Depicted are subjective ratings (means and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and test phase (B) of the whole sample (N = 22) and during the test phase of the learner subgroup (C; N = 11) and non-learner subgroup (D; N = 11). The dashed line depicts the pain threshold (VAS = 40).

Subjective decay of the nocebo response

For an illustration of the subjective ratings after the self-regulation procedure (VAS_2), please refer to Figure SIII in the Appendix.

To explore how the subjective rating developed over time within trials, the difference between VAS_2 and VAS_1 was calculated. In the learning phase, the difference between the ratings was larger for trials cued with CS+ compared to CS–, indicating that the subjects showed temporal summation during the trials in response to the moderately painful stimulation (Figure 9A; main effect 'CS': $F(1, 512.3) = 45.86$, $p < .001$). The degree of temporal summation decreased over time (main effect 'time': $F(14, 566) = 2.23$, $p = .006$), which was similar for trials cued

with either CS (interaction effect 'CS' x 'time': F(14, 512.3) = 1.41, p = .144). This shows that although subjects showed temporal summation during each trial they showed habituation across trials, depicting opposing processes on the short- and long-term. A subjective decay, as in Study 1, was not apparent.

In the test phase, the difference in subjective ratings was not different for CS+ and CS– when considering the whole sample (main effect 'CS': F(1, 206.8) = 0.16, p = .694; main effect 'time': F(6, 235.2) = 0.82, p = .554; interaction effect 'CS' x 'time': F(6, 206.8) = 1.69, p = .124; Figure 9B) or restricting the analysis to the learner subgroup (main effect 'CS': F(1, 102.3) = 2.42, p = .123; main effect 'time': F(6, 113.3) = 0.59, p = .737; interaction effect 'CS' x 'time': F(6, 102.3) = 1.22, p = .302). This means that a subjective decay of the nocebo response, as shown in Study 1, could not be demonstrated.

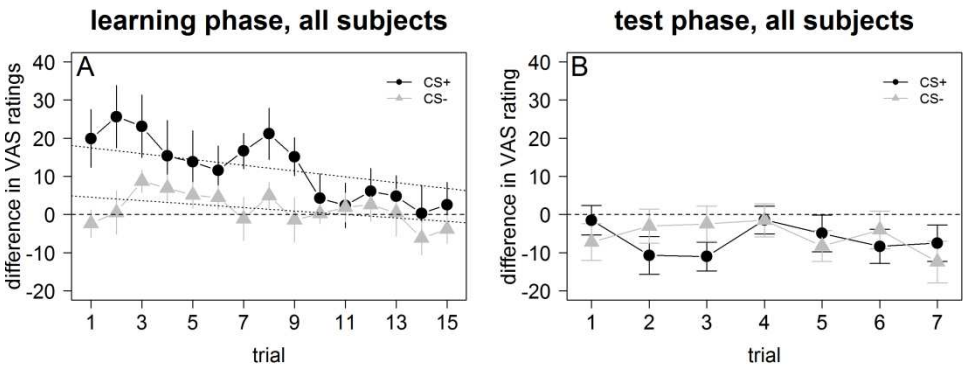

Figure 9: Subjective decay of the conditioned nocebo response ($VAS_2 – VAS_1$) throughout the experiment. Depicted are the differences in subjective ratings (means and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and test phase (B) of the whole sample (N = 22).

Self-regulation procedure

Corresponding to the subjective ratings, no nocebo effect in the behavioral response emerged in the test phase, considering the whole sample (main effect 'CS': F(1, 248.3) = 0.72, p = .397; main effect 'time': *F*(6, 288.3) = 0.45, *p* = .844; interaction effect 'CS' x 'time': *F*(6, 248.3) = 0.94, *p* = .464; Figure 10B). Contrary to our hypothesis, however, restricting the analysis to the learner subgroup, no

conditioned effect occurred (main effect 'CS': F(1, 129.9) = 0.18, p = .668; main effect 'time': *F*(6, 151) = 0.48, p = .822; interaction effect 'CS' x 'time': *F*(6, 129.9) = 1.4, p = .218). These results indicate that a decay of the conditioned nocebo response in the behavioral measure could not be shown.

In the learning phase of the experiment, increased temporal summation for moderately painful compared to mildly painful trials was observable in the behavioral response (Figure 10A; main effect 'CS': F(1, 502.6) = 36, p < .001). The degree of temporal summation decreased over time (Figure 10A; main effect 'time': F(14, 574) = 2.34, p = .004) but this was similar for both trials cued with CS+ and CS– (interaction effect 'CS' x 'time': F(14, 502.6) = 0.9, p = .556).

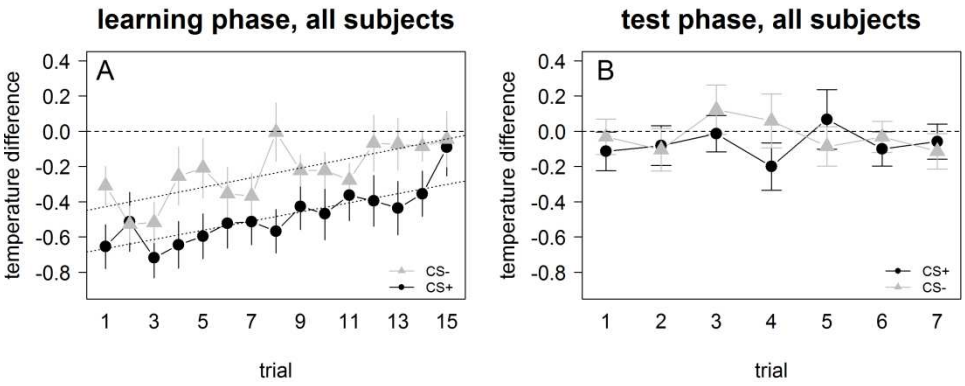

Figure 10: Behavioral responses throughout the experiment. Illustrated are the self-adjusted temperature changes (means and standard errors of mean) of the single trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and the test phase (B; N = 22).

Autonomic indicators: heart rate and parasympathetic heart control

The mean trial length on which heart rate and heart rate variability are based was 1:13:24 min. (SD = 11.46 sec).

Trials cued by CS+ or CS– did not show differences in heart rate in the learning phase (CS+: M = 76.4, SD = 9.07; CS–: 76.1 SD = 9.02) or the test phase (CS+: M = 75.1 SD=9.05; CS–: M = 75.3 SD = 9.19). In the test phase, no significant differences between trials cued with CS+ (M = -0.13, SD = 1.32) and CS– (M = -0.28, SD = 1.14) emerged, neither within the whole sample nor within a subgroup.

Post hoc analyses, however, showed that the learners and non-learners differed in their autonomic responses during the learning and test phase. The learner subgroup had increased heart rate compared to the non-learners and reduced HRV in almost all reported parameters (Table 1), indicating reduced parasympathetic activity in both the learning and the test phase.

Table 1: Heart rate and heart rate variability parameters for learners (N = 8) and non-learners (N = 10).

	learning phase			**test phase**		
parameter	**learners**	**non-learners**	**t-test**	**learners**	**non-learners**	**t-test**
	M, SD	**M, SD**	**T, p**	**M, SD**	**M, SD**	**T, p**
HFREQ	78	74.9	**4.1**	77.7	73.2	**4.4**
	8.11	9.51	**<.001**	8.03	9.41	**<.001**
nNN50	12.4	14	-1.8	11.9	15.1	**-.25**
	10.79	10.96	.081	10.13	11.59	**.011**
pNN50	17.5	20.4	**-2**	17	22.7	**-2.9**
	15.57	17.34	**.045**	14.91	18.97	**.003**
RMSsd	35.1	39.9	**-3 3**	35.3	43.5	**-3.9**
	17.64	16.38	**.001**	17.56	19.25	**<.001**
RRms	782.1	819.7	**-4.4**	785.8	839.4	**-4.7**
	85.6	113.16	**< .001**	86.63	114.61	**< .001**

M, mean; SD, standard deviation; T, T-value, p, p-value; HFREQ, heart rate frequency; nNN50, proportion of the number of pairs of successive beat-to-beat intervals that differ by more than 50 ms divided by total number of beat-to-beat intervals; pNN50, percentage of differences of subsequent beat to beat intervals greater than 50 ms; RMSsd, root mean square of successive differences; RRms, time of beat-to-beat interval in ms.

Contingency awareness

All subjects were able to distinguish the two CS (32°C and 36°C pulse), as verified prior to the conditioning task. Seven subjects out of 21 (33.3 %) were able to describe the contingency between the CS and the US in the interview after the experiment and three out of these seven (42.9 %) belonged to the learner subgroup. Of the fourteen subjects who did not become contingency aware, eight (57.1 %) belonged to the learner subgroup, indicating that contingency awareness was not a necessary condition for successful conditioning of the subjective nocebo response. The positive intercept in the regression analyses (intercept = 1.3)

confirmed that the nocebo response was independent of contingency awareness, as expected (Figure 11). Further, the negative slope (slope = -0.6) revealed that contingency awareness was not helpful for the nocebo effect.

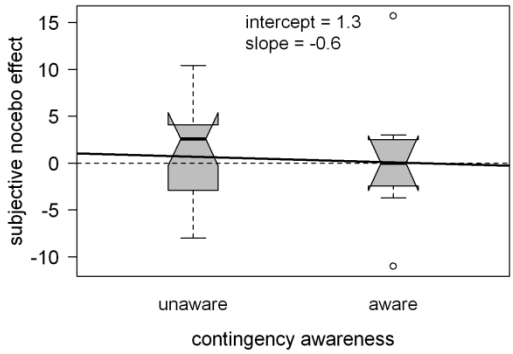

Figure 11: Regression of subjective nocebo effect with contingency awareness. Participants were categorized as aware or unaware of the contingency between CS and US. The notch of the box plots displays the median and the length of the notched bar displays a confidence interval around the median.

Anxiety, motivational style, and suggestibility

A negative correlation of trait anxiety with the subjective nocebo response ($r = -0.466$, $p = .033$) emerged, after exclusion of an outlier from the analysis (Figure 12). Contrary to our hypothesis, learners were habitually less anxious ($M = 32$, $SD = 3.5$, $N = 10$) compared to non-learners ($M = 37.4$, $SD = 7.28$, $N = 11$). No correlations of the nocebo response with state anxiety ($r = -0.076$, $p = .738$), BIS score ($r = -0.45$, $p = .843$), sum score or subscales of BAS (all $p > .104$), or suggestibility measures ($r = -0.099$, $p = .66$) were detectable and no differences between learners and non-learners appeared.

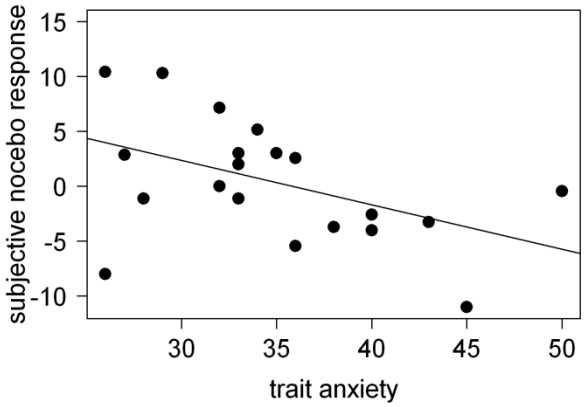

Figure 12: **Negative correlation of trait anxiety and the subjective nocebo response (r = -0.466, p = .033, N = 21).**

Discussion

Nocebo conditioning led to a subjective nocebo response within the painful range (i.e., nocebo-hyperalgesia), however, only in part of the sample, in line with previous studies. These learners were presented with significantly higher stimulus intensities and evaluated the stimuli as more painful during the test phase, but not during the learning phase of the experiment. They further had an increased heart rate and reduced HRV parameters, compared to the non-learners. Contingency awareness was independent from and proved to be not helpful for the learning of the subjective nocebo response. A decay of the conditioned response over time could not be demonstrated within the subjective or behavioral measures, in contrast to Study 1. Successfully conditioned subjects had lower scores on trait anxiety and the less anxious subjects were the greater was the subjective nocebo response. Motivational style and suggestibility were not correlated with the subjective nocebo response.

By partially replicating results from Study 1, we could demonstrate that classical conditioning without conscious expectation is sufficient to induce a nocebo response. A number of possible confounding variables was explored, but a covariation with the nocebo response was not found. In contrast to Study 1, subjective ratings in the test phase of this experiment were above the pain

threshold, indicating nocebo-hyperalgesia. Further, significantly different stimulus intensities albeit equivalent subjective ratings (as ensured by the determination of US intensities and comparison of the subjective ratings during the learning phase) were applied in the learner and non-learner subgroup. However a lack of difference in the subjective ratings does not mean that stimuli of different temperatures are equivalent at the neurophysiological, neural or behavioral level. This finding suggests that nocebo-hyperalgesia might be more readily induced with higher stimulus intensities.

Most subjects remained contingency unaware throughout the experiment despite increased differentiation of the stimuli compared to Study 1. The proportion of subjects who recognized the contingency was even lower than in Study 1, which might be due to greater distraction by the increased painfulness of the stimuli or the employed conditioning design, which was a trace conditioning in this case. However, replicating results from Study 1, contingency awareness neither was necessary for the subjective nocebo effect to occur nor was it beneficial, showing again that a nocebo response can be learned without explicit expectations.

Contrary to results in the context of the placebo effect (Wickramasekera, 1980; De Pascalis et al., 2002; Schweinhardt et al., 2009), suggestibility and motivational style did not correlate with the nocebo effect in this study, further emphasizing that the nocebo effect is not just a reversed placebo effect (Benedetti et al., 2006; Colloca et al., 2008; Kong et al., 2008). Trait anxiety strongly correlated with the nocebo effect in this study, however, contrary to our hypothesis, the more habitually anxious the subjects were the smaller the nocebo effect appeared. A similar result was reported by Colloca et al. (2010) who found a negative correlation of the nocebo response with trait (and state) anxiety after a short conditioning procedure with non-painful stimuli and a positive correlation after conditioning with painful stimuli. Compared to Colloca's et al. (2010) study, our procedure was of medium length (15 trials compared to 5 trials in the short and 20 trials in the long conditioning procedure). Although in Study 1, the employed stimuli were non-painful, we did not observe a relation between anxiety and the subjective nocebo response. Further, the learners, who showed less anxiety than the non-learners, rated the presented stimuli as above the pain threshold and reported significantly more pain than the non-learners. Although the learners received higher stimulus intensities, trait anxiety did not correlate with stimulus intensity ($r = -0.308$, $p = .175$), indicating that the negative correlation between anxiety and the nocebo effect cannot be attributed to differences in stimulus intensity. Other studies that employed painful stimulation did not find correlations

with anxiety (Schmid et al., 2013; Vogtle et al., 2013). These unexplained differences in the role of anxiety might partly be due to variations in experimental designs. Unquestioned, knowledge on characteristics of nocebo responders is important because it can be utilized in the clinical context. For example, subjects that are identified as being highly susceptible for nocebo effects could be administered a special treatment (e.g., additional information) in order to prevent the development of nocebo-related adverse effects. For this reason the relations between the nocebo response and anxiety besides other potential personality traits or characteristics should be further explored.

As autonomic measures, we investigated heart rate and time domain parameters of HRV because the trial length was too short to explore parameters from the frequency domain. Changes in HRV can reflect reactivity of the autonomic system in response to environmental and experimental conditions (Berntson et al., 1997; Porges, 2007) and especially pain (Koenig, Jarczok, Ellis, Hillecke, & Thayer, 2013; Loggia, Juneau, & Bushnell, 2011). Accordingly, placebo-hypoalgesia has shown to be accompanied by reduced heart rate and increased sympathetic components (Aslaksen & Flaten, 2008; Pollo et al., 2003). As muscarine blockade left the placebo-hypoalgesia unaffected the authors concluded that parasympathetic systems were not involved (Aslaksen & Flaten, 2008; Pollo et al., 2003). Although in the present study, a nocebo effect in the autonomic measures on single trial level (i.e., difference between trials cued by CS+ and CS–) was not detected, autonomic indices were different between learners and non-learners. Evidence shows that heart rate responses are more closely related to subjective sensation than to stimulus intensities (Möltner, Hölzl, & Strian, 1990), which is in line with the observation in this study that learners evaluated the presented stimuli as more painful during the test phase of the experiment. The fact that learners displayed a pattern of increased heart rate and decreased HRV already in the learning phase, suggesting decreased parasympathetic activation, might indicate that the learner were more stressed by the pain stimulation compared to the non-learners and as a consequence learned more successfully, as evidence shows that stress can enhance conditioning (Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007; Shors, Weiss, & Thompson, 1992).

In contrast to Study 1, the learner subgroup did not display a decay of the nocebo response in the subjective and behavioral measures. This might be due to different aspects which differentiate this study from the previous one. For example, the temperatures employed in this study were higher, which might have promoted effects of temporal summation and perceptual sensitization, interfering with the

decay of the conditioned response. It could be the case that it takes longer for the pain system to “recover” with stronger pain stimulation. Another possibility is that the conditioning was not strong enough (as indicated by the lower number of successfully conditioned subjects compared to Study 1). Assuming that effects of descending inhibition might play a role in the pattern observed in the behavioral and subjective measures of decay of the conditioned response and that descending inhibition might be triggered by the CS, it could be concluded that weaker conditioning leads to less descending inhibition, preventing the occurrence of decay indicated by adaption.

The newly introduced booster trials (presenting CS+ followed by the moderately painful stimulus three times across the test phase) might provide an explanation for the generally less pronounced conditioning success compared to Study 1. They had the purpose to increase the conditioned nocebo effect and prevent quick extinction. However, according to the theory of adaptation-level (Helson, 1947), a stimulus far above the stimulus range (i.e., during a booster trial) will change the adaption level. As a consequence, the discrimination of stimuli at previous stimulus range (i.e., the mildly painful intensity cued with CS+ or CS– during the test phase) deteriorates. Thus, the booster trials might have diminished the conditioning effect. In addition to that, one can assume that effects of distraction are stronger the more painful the employed stimuli are. Consequently, with the increased intensity level compared to Study 1, successful conditioning might have been more difficult to achieve.

In conclusion, the present study shows that subjective nocebo-hyperalgesia was successfully conditioned in part of the sample. Learners compared to non-learners were lower in trait anxiety, developed increased heart rate and decreased HRV, and subjectively rated the stimuli as more painful during the test phase. Contingency awareness was independent from learning and neither motivational style nor suggestibility were correlated with the nocebo response. Presumably due to several modifications compared to Study 1 (e.g., booster trials, trace conditioning, higher stimulus intensities) the conditioning effect was less pronounced and a decay of the conditioned response over time could not be shown in the subjective and behavioral measures.

3. Neural Correlates of Pain Sensitization Induced by Uncontrollability

Introduction

Would you rather remove a splinter from your finger yourself or let somebody else do it? Many people would choose the former, illustrating the empowerment of having control over pain (Bowers, 1968). Conversely, not having control over pain is particularly relevant from a clinical point of view: it leads to feelings of helplessness (Burger, 1980; Maier & Watkins, 2005) and contributes to chronic pain and co-morbid depression (Flor et al., 1990; Müller, 2011). For improving pain management and therapy, a better understanding of pain uncontrollability is deemed essential (Borckardt et al., 2011; Haythornthwaite et al., 1998).

Experimental work confirms that controllable pain stimuli are perceived as less intense than uncontrollable stimuli (Arntz & Schmidt, 1989; Müller, 2011). Human as well as animal studies point to an important role of the prefrontal cortex in mediating analgesic effects of (perceived) control over pain (Amat et al., 2005; Borckardt et al., 2011; Wiech et al., 2006). In contrast, it is currently unknown which brain areas drive the augmentation of pain when the stimulus is uncontrollable.

Candidate brain regions should show increased activation when pain is uncontrollable compared to when it is controllable. Several brain regions associated with pain processing, including ACC, insula, SI, SII, amygdala, PAG, and PFC (Helmchen et al., 2006; Mohr et al., 2005; Salomons et al., 2004; Salomons et al., 2007; Wiech et al., 2006) have been identified to fulfill this criterion. However, activation in many of these regions scales linearly with perceived pain intensity (Coghill, Sang, Maisog, & Iadarola, 1999; Loggia et al., 2012; Seminowicz & Davis, 2007), and signal increases in such areas might therefore reflect the increased pain perception when pain is uncontrollable. But three regions, namely PAG, amygdala, and perigenual ACC (pACC), that have been reported to show increased activation when pain is uncontrollable, are interesting candidates because their stimulus-response curves do not typically follow a simple linear relationship (Bornhovd et al., 2002; Loggia et al., 2012; Neugebauer & Li, 2002; Porro, Cettolo, Francescato, & Baraldi, 1998). Furthermore, PAG, amygdala, and pACC are known to play

important roles for endogenous pain modulation (Bingel, Lorenz, Schoell, Weiller, & Büchel, 2006; Neugebauer, Li, Bird, & Han, 2004; Vanegas & Schaible, 2004). The PAG is a major relay site of the descending pain modulatory system and involved in pain facilitation (Gwilym et al., 2009; Porreca et al., 2002; Yoshida et al., 2013). The amygdala plays a key role in fear and threat processing, also in the context of pain (Neugebauer et al., 2004; Phelps & LeDoux, 2005), and it has been hypothesized that a lack of control increases the threatening value of pain (Arntz & Schmidt, 1989; Bowers, 1968). Lastly, the pACC is involved in different types of cognitive-emotional modulation of pain (Bingel et al., 2006; Wiech, Ploner, & Tracey, 2008) and has been shown to exhibit heightened activation when pain is provoked in sensitized states (Lorenz et al., 2002).

Here, we tested the hypothesis that a circuitry encompassing PAG, amygdala, and pACC mediates pain augmentation by uncontrollability, using fMRI. An experimental design allowing the dynamic investigation of pain processing and sensitization was used to differentiate between brain areas reflecting increased pain processing and a network of brain structures driving the increased pain perception.

Methods

Subjects

Inclusion criteria included age between 18 and 40 years and good health. Exclusion criteria comprised the presence of any chronic pain condition, presence or history of significant neurological or psychiatric disease; presence of any significant medical condition; regular consumption of alcohol or recreational drugs; recent use of any pain medication; regular or frequent night shift work, presence of any sleep disorders. The study was approved by the local ethics committee, and written informed consent was obtained from all subjects. Subjects were compensated for their participation.

Three subjects were excluded because the painful stimulus was not evaluated as painful during more than 50 % of the pain trials. The final sample consisted of 23 volunteers (13 males; 1 left-handed), aged 19–30 years (mean 24.2 ± 3.57 years).

General procedure

Each subject underwent two sessions: a familiarization session and an fMRI session, spaced by one to three days.

Familiarization session

The study was explained to the subject and after obtaining informed consent, subjects were familiarized with the stimuli, the tasks, and the rating scales and their pain thresholds were assessed. Cutaneous heat stimuli were delivered using a 30 x 30 mm contact thermode (Pathway, Medoc Ltd Advanced Medical System, Israel). Stimulus intensities were individually determined (see below); but temperatures above 50 °C were not allowed for safety reasons. A vertically oriented visual analog scale (VAS) anchored with 0 (*no sensation*), 40 (*just painful,* defined as the pain threshold), and 100 (*most intense pain tolerable*) was used to rate non-painful and painful sensations. This VAS has been shown to possess linear properties (Lautenbacher et al., 1992). Pain tolerance testing was performed so that the subjects experienced a sensation that corresponded to the upper end of the VAS, thereby providing a perceptual anchor. For this, the thermode was applied to the subject's forearm and the temperature slowly increased (rise rate 0.5 °C/s) from 32 °C until the subject stopped it by pressing a button on the response unit. This procedure was repeated twice with a rise rate of 1.5 °C/s.

To assess the pain threshold, a series of stimuli was applied to the testing site to be used during the fMRI, i.e., the subject's non-dominant thenar eminence. After each stimulus, the subject indicated the most intense sensation on the VAS. The baseline temperature was 36 °C and the first target temperature was 39 °C (rise rate of 2.5 °C/s, stimulus duration 5 s). The subsequent target temperatures each increased by 1 °C until the subject rated a stimulus as 'just painful'. Subsequently, five more temperatures around this initial painful temperature were applied to determine the pain threshold. The resulting average pain threshold was 44.2 °C (SD = 1.42 °C), consistent with the literature (Rolke et al., 2006).

The temperature to be used as initial temperature in the controllable pain trials was based on the individual's pain threshold by adding 1.5 °C; however, adjustments were made if this temperature was not rated as moderately painful or did not fall between 45.5 °C and 48°C (mean temperature = 47 °C, SD = 0.78 °C). For the controllable warm trials, the initial temperature was 39 °C or 40 °C,

depending on the subject's rating in the familiarization session (mean temperature = 39.5 °C, SD = 0.5 °C).

Experimental design

The study followed a within-subject yoked-control design with two within factors (condition 'controllable' vs. 'uncontrollable' and stimulation intensity 'moderate pain' vs. 'warmth'). The sequence of stimulation intensities was pseudo-randomized with the same order across subjects. In each functional scan, the first 12 trials were controllable, followed by 12 uncontrollable trials to avoid frequent switching between the two conditions so that subjects would not be confused with respect to the task to be performed in a specific trial. In the controllable condition, subjects performed a temperature regulation task (Becker et al., 2011; Hölzl et al., 2005; Kleinböhl et al., 1999). After the thermode had reached the target temperature, the subject had to keep his/her sensation of the stimulus constant by antagonizing any perceived temperature change with the response unit by regulating the temperature down or up using the left or right mouse button, respectively. If the sensation had not changed, subjects had to press the middle mouse button to control for motor responses. Because the temperature only changed when the subject regulated it, any change perceived by the subject was due to temporal summation or adaption. A flashing arrow reflected the subject's response and served as visual feedback to minimize unspecific differences to the uncontrollable condition. After 20 s the temperature returned to baseline. In the uncontrollable condition, the temperature profiles of the previous controllable trials were replayed (yoked control). The subject had to continuously rate the sensation on the VAS, which was projected onto the screen. If the sensation had not changed, subjects had to press the middle mouse button. During both tasks a green square flashed every two seconds prompting the subject to give a response, controlling for response rate. The inter-trial-interval was 20 s.

Questionnaires

The IPC scale (Levenson, 1981) was used to identify the locus of control beliefs (subscales: internal, powerful others, and chance). Twenty subjects completed this questionnaire. All subjects completed the State-Trait Anxiety Inventory (STAI; Spielberger, 1970) indicating their level of state and trait anxiety.

FMRI session

At the beginning of the fMRI session, subjects were reminded of the tasks and stimulus intensities were adjusted if necessary. FMRI data acquisition was performed in two functional scans, separated by an anatomical scan.

Stimulus presentation was controlled by a personal computer using Eprime software (Psychology Software Tools Inc., Pittsburgh, USA). The display was back-projected onto a screen, visible via a mirror that was mounted on the head-coil of the MRI scanner. A computer mouse with three buttons was modified in-house to ensure MR-compatibility and allow for communication with the software. The mouse served as response unit so that the subjects could perform the experimental tasks.

FMRI data acquisition

Imaging data were acquired on a 3 T Siemens TRIO MRI scanner at the McConnell Brain Imaging Center, Montreal Neurological Institute (MNI). A multi-slice, gradient-echo EPI sequence covering the whole brain was used for functional scans (TR = 2.62 s, TE = 30 ms, flip angle = 90 degree, 44 interleaved, 3.5-mm thick axial slices (parallel to the AC-PC line), field of view (FoV) 224 mm x 224 mm, matrix 64 x 64, resulting in an in-plane resolution of 3.5 x 3.5 mm^2, 441 image volumes). The first two images were discarded to allow steady state magnetization. Field maps were obtained using a gradient echo sequence (TE = 20 ms, 0.47 ms dwell time, FoV and matrix identical to EPI). High-resolution, anatomical T1-weighted images (RF spoiled, pre-scan normalized MPRAGE sequence, TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, flip angle = 9 degree, FoV 192 mm x 256 mm x 256 mm, matrix 192 x 256 x 256, hence voxel size: 1 mm^3) were acquired for all subjects for co-registration purposes.

Statistical analysis of behavioral data

Mean values and standard deviations were calculated for the self-adjusted temperature changes and the subjective ratings of the intensity. Two-sided t-tests were used to investigate whether the temperatures, respectively VAS ratings, at the end of the trial differed from the value at the beginning of the trial. A linear mixed model was used to test whether the VAS ratings changed over subsequent trials. The IPC scores were correlated with the psychophysical data using Pearson's

correlations. Alpha = 0.05 was used as significance level. Statistical tests were performed with PASW Statistics 17.0.3. Figures were prepared with R 2.11.1.

Statistical analysis of fMRI data

All image processing and statistical analysis was performed using the software package FSL 4.18 (FMRIB's Software Library; http://www.fmrib.ox.ac.uk/fsl; Smith et al., 2004). Out of 1104 trials in total, 19 trials were excluded due to missing data caused by technical problems, such as thermode malfunction. Eleven trials were shortened due to deviations from the specified temperature profile.

Subject level analysis. The following preprocessing steps were applied to each functional dataset: denoising using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) within FEAT (FMRI Expert Analysis Tool), spatial smoothing (Gaussian kernel, full width at half-maximum: 5 mm), motion correction, and temporal highpass filtering (Gaussian-weighted least-squares straight line fitting with sigma = 100 s). Susceptibility-related distortions were corrected using FSL field map correction routines.

A general linear model (GLM) was applied to each functional dataset, modeling the four conditions (controllable-painful, controllable-nonpainful, uncontrollable-painful, uncontrollable-nonpainful). In a second GLM, total temperature changes over the course of individual controllable trials (excluding the stimulus rise time), moment-to-moment temperature changes reflecting temperature changes every second (controllable trials), intensity ratings over the course of individual uncontrollable trials, and moment-to-moment changes of intensity ratings (uncontrollable trials) were included as explanatory variables in addition to the four explanatory variables for condition. To account for the time subjects needed to 'catch up' with the initial temperature increase of the thermode in the uncontrollable condition, the first 4 seconds of the 20 second stimulation period were not included in the regressors (please refer to Figure 13). This model allowed identifying a) brain activation correlating with sensitization in the respective conditions, and b) activation that is neither explained by condition nor by increases in pain perception and should thereby reveal brain regions that drive the sensitization (DRIVE contrast). Regressors were convolved with a gamma hemodynamic response function and the first temporal derivatives were included. Multicollinearity between the regressors was ruled out by correlation analyses. Temperature rise and fall times, motion outliers, and time series for cerebrospinal fluid and white matter were included in the models as nuisance variables. Voxel-

wise parameter estimates (PEs) were derived using the appropriate contrasts. Individual's functional images were registered to the 152 template in MNI standard space using linear (FLIRT, Jenkinson, Bannister, Brady, & Smith, 2002) and non-linear transformations (FNIRT, warp resolution=10 mm). These transforms were applied to the subject-level statistical images.

Group level analysis. The parameter estimates and the corresponding estimates of the variance from the two functional scans of each subject were merged in a second level fixed effects analysis. Third level analyses were performed using a mixed-effects model, implemented in FLAME (Beckmann, Jenkinson, & Smith, 2003). Statistical inference was based on the whole brain with a voxel-based, cluster-forming threshold of Z = 2.3 and correction for spatial extent according to Gaussian random field theory (Worsley, Evansy, Marretty, & Neeliny, 1992) at a cluster-level of $p < 0.05$. For the second GLM, constructed to identify areas that drive uncontrollability-induced pain increases, we employed additional region of interest (ROI) analyses, in particular because the hypothesized regions (PAG, amygdala, pACC) are small. ROIs of the brainstem, bilateral amygdala, and pACC were defined anatomically on the MNI-152 template by means of the Harvard-Oxford Cortical and Subcortical Atlases as implemented in fslview 4.18 and transformed in each individual's functional space. A voxel-based threshold of Z = 1.6, cluster-level corrected at $p < 0.05$ was used for statistical inference of ROI analyses.

Localization of activation was achieved by inspection of group activation maps overlaid onto the average high-resolution image of the subjects. Coordinates are given in MNI space.

Results

Behavior

In the controllable condition during painful trials, subjects regulated the temperature down in order to keep their sensation constant (Figure 13A), which indicated that temporal summation occurred over the course of the 20 second-long trials (temperature difference between end and beginning of the trial: M = -0.7 °C, SD = 0.398 °C; T = 8.41, $p < 0.001$). During warm trials, subjects on average

increased the temperature, i.e., they adapted to the stimulus (M = 0.19 °C, SD = 0.305 °C; T = -3, p = 0.007, Figure 13B).

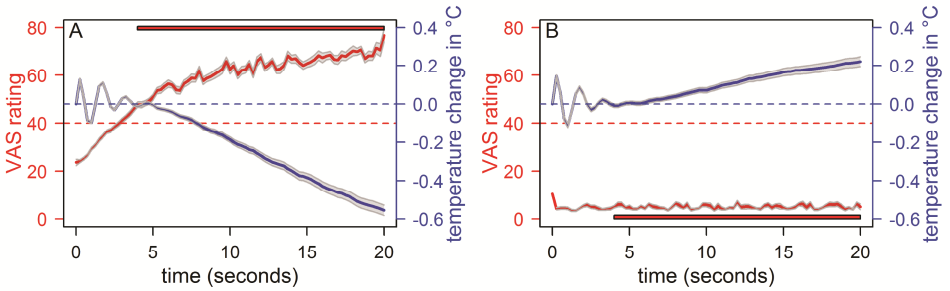

Figure 13: Psychophysical data of painful (A) and non-painful trials (B). Illustrated are the temperature courses in blue, averaged across trials and particpants, and the Visual Analog Scale (VAS) ratings, equally averaged across trials and particpants, in red. For better illustration, we downsampled the displayed data (interval length = 250 ms). Grey ribbons represent the standard error of the mean. Because increasing ratings at the beginning of the uncontrollable trials do not reflect increasing pain sensation (the subjects needed time to adjust their rating after the thermode had reached the target temperature), the first 4 seconds of the uncontrollable trials were excluded. The red bars indicate the time segment that was included in the comparison of intensity ratings between end and beginning of the trial as well as as pain rating regressors in the fMRI analysis of the uncontrollable trials (see Methods). The red dotted line depicts the "just painful" anchor of the VAS. The blue dotted line markes adaption (values > 0) and temporal summation (values < 0) for the controllable trials. The initial variations in temperature were due to fluctuations of the thermode.

In the uncontrollable condition, subjects were administered the identical nociceptive input as in the controllable condition by replaying the self-adjusted temperature time courses of the controllable trials. In line with the notion that uncontrollability increases pain, they rated the sensation as continuously getting more painful during pain trials (rating difference between end and beginning of trial: M = 23.2, SD = 9.22; T = 10.95, p < 0.001; Figure 13A), despite that they had regulated the temperature time courses to produce constant sensations in the controllable condition. During warm trials, the ratings remained stable throughout the trial (M = 0.38, SD = 1.06; T = 0.51, p = 0.62; Figure 13B) and thus matched the perceived sensation in the controllable condition. These results indicate that the effects of uncontrollability on the perception of thermal stimuli are specific for painful sensations.

The design required that the uncontrollable trials were presented after the controllable trials. But sensitization across trials can be ruled out as an alternative explanation for the increased pain in the uncontrollable trials because pain ratings did not increase across trials (please refer to Figure SIV in the Appendix; interaction effect 'Trial' x 'Intensity': $F(5, 514) = 0.7$, $p = 0.626$).

Locus of control and anxiety scores

Regarding individual control beliefs, the extent of temperature change in the controllable painful condition correlated positively with the "powerful others subscale" of the IPC locus of control scale ($r = 0.59$, $p = 0.012$). This indicates that the more a subject believed that his/her life is controlled by others, the more he/she showed temporal summation. Neither trait ($M = 36.4$, $SD = 9.74$) nor state anxiety scores ($M = 29.1$, $SD = 8.07$) correlated with the behaviorally indicated change in sensation in the controllable or uncontrollable conditions (all p-values > 0.46).

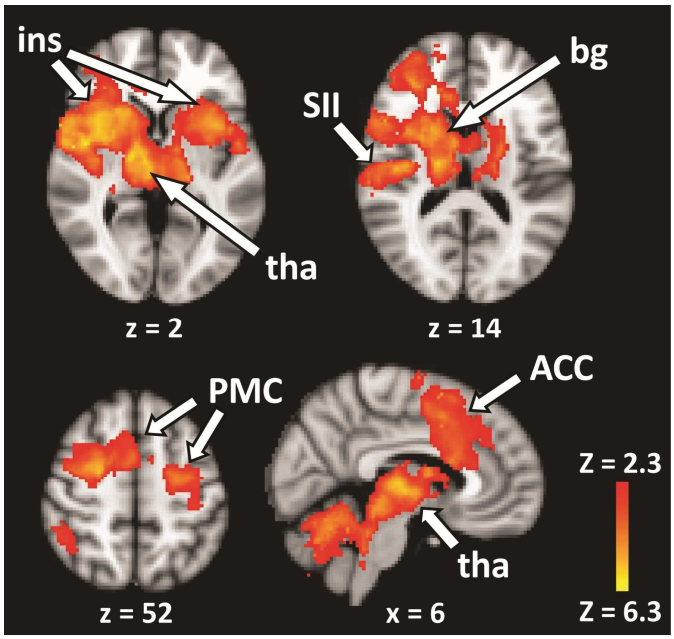

Figure 14: Brain responses to painful vs. warm stimulation, irrespective of the task (controllable and uncontrollable painful > controllable and uncontrollable warm). Increased activation in pain processing brain areas: bilateral insula (ins), thalamus (tha), basal ganglia (bg), secondary somatosensory cortex (SII), premotor cortex (PMC), and anterior cingulate cortex (ACC). Statistical inference was based on a voxel-based threshold

of Z = 2.3 and a cluster-based threshold of p = 0.05, corrected for multiple comparisons across the whole brain. The activation map is overlaid on the MNI-152 template. Images are displayed in radiological convention, i.e., right side of the brain is on the left. For details see Supplementary Table I

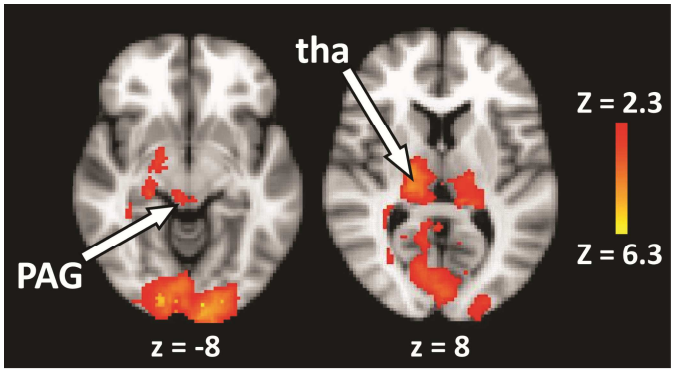

Figure 15: Brain responses to uncontrollable painful stimulation compared to controllable painful stimulation (uncontrollable painful > controllable painful). Increased activation was detected in periaqueductal gray (PAG) and bilateral thalamus (tha). Statistical inference was based on a voxel-based threshold of Z = 2.3 and a cluster-based threshold of p = 0.05, corrected for multiple comparisons across the whole brain. The activation map is overlaid on the MNI-152 template. Images are displayed in radiological convention, i.e., right side of the brain is on the left. For details see Supplementary Table II.

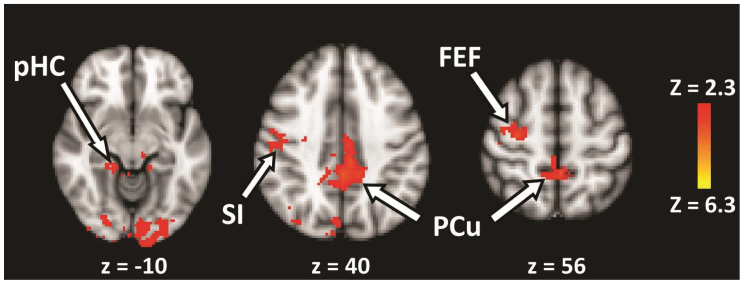

Figure 16: Brain activation correlating with temporal summation in the controllable condition. Remarkably, no activation in pain processing areas correlated with temporal summation in the controllable condition. Statistical inference was based on a voxel-based threshold of Z = 2.3 and a cluster-based threshold of p = 0.05, corrected for multiple comparisons across the whole brain. The activation map is overlaid on the MNI-152 template. Images are displayed in radiological convention, i.e., right side of the brain is on the left. For details see Table S4. pHC, parahippocampus; SI, primary somatosensory cortex; PCu, precuneus; FEF, frontal eye field. For details see Supplementary Table III.

Brain activation in response to painful stimulation

When pain trials were compared to warm trials, activations were found in typical pain processing areas including bilaterally insula, ACC, SII, premotor cortex, basal ganglia and thalamus (Figure 14, Supplementary Table I). Activations for the controllable and uncontrollable condition were largely similar albeit PAG activation was only found for the uncontrollable condition, and was significant in the contrast $Pain_{uncontrollable}$ minus $Pain_{controllable}$. Similarly, thalamic activation was significantly stronger during uncontrollable pain than during controllable pain, mainly in the right hemisphere (Figure 15, Supplementary Table II).

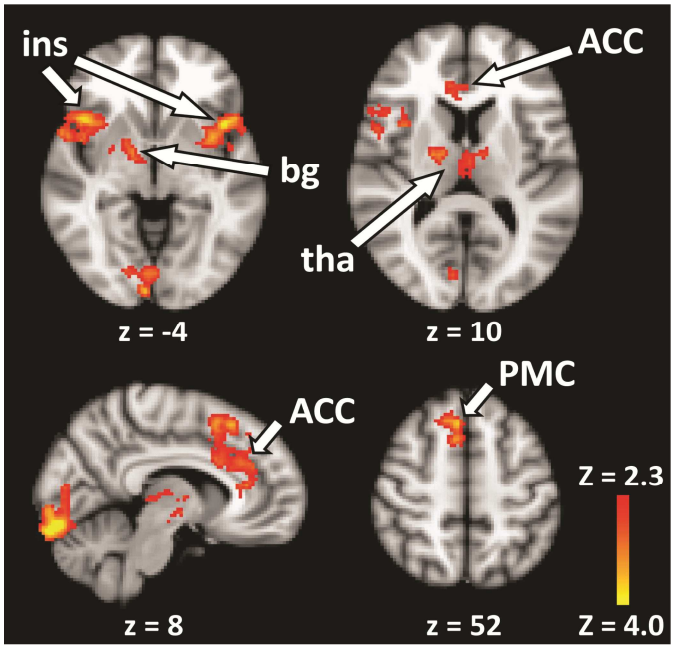

Figure 17: The subjective ratings of the uncontrollable painful condition correlated with activation in pain processing areas: insula, basal ganglia (bg), thalamus (tha), anterior cingulate cortex (ACC), premotor cortex (PMC). This activation is controlled for activation unrelated to subjective perception (constant regressor) and activation that might be driving the increases in pain perception (moment-to-moment changes of pain perception). Statistical inference was based on a voxel-based threshold of Z = 2.3 and a cluster-based threshold of p = 0.05, corrected for multiple comparisons across the whole brain. The activation map is overlaid on the non-linear Montreal Neurological Institute (MNI)-152 template. Images are displayed in radiological convention, i.e., right side of the brain is on the left. For details see Supplementary Table IV.

Brain activation associated with temporal summation in the controllable condition

Activation in none of the pain-processing regions correlated with the down-regulation of the temperature, indexing temporal summation, in the controllable pain trials. Areas in which activation correlated with the temperature time course included the precuneus, the parahippocampus, and an area in vicinity to the frontal eye field (Figure 16, Supplementary Table III), which is interesting because it might reflect the cognitive and attentional demand of the task.

Brain activation associated with the additional sensitization in the uncontrollable condition

The increases in pain perception in the uncontrollable condition, indexed by subjective ratings, correlated with activation in typical pain processing regions, including bilateral insula, rostral and mid ACC, supplementary motor cortex (BA8), bilateral anterior and medial thalamus, lateral thalamus mainly contralateral to stimulation site, lentiform nucleus, and occipital lobe (Figure 17, Supplementary Table IV).

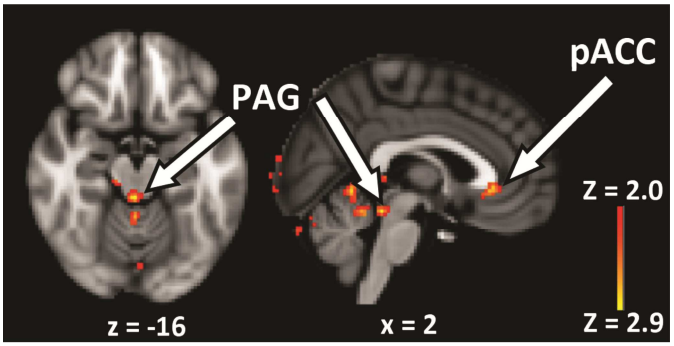

Figure 18: Activation in perigenual anterior cingulate cortex (pACC; x = 2, y = -36, z = -16, Z score peak = 2.88, cluster size = 12 voxels) and periaqueductal gray (PAG; x = -2, y = 30, z = -2, Z score peak = 3.3, cluster size = 2 voxels) correlated with moment-to-moment changes in pain perception in the uncontrollable painful condition. This activation is controlled for activation unrelated to subjective perception (constant regressor) and activation correlating with pain ratings. Statistical inference was based on a region of interest (ROI) analysis with a voxel-based threshold of Z = 1.6 and a cluster-based threshold of p = 0.05, corrected for multiple comparisons across the ROI. The activation is overlaid on the non-linear MNI-152 template. Images are displayed in radiological convention, i.e., right side of the brain is on the left.

Brain regions driving additional sensitization in the uncontrollable condition

To identify brain regions that drive the augmented pain perception in the uncontrollable condition, activation correlating with the moment-to-moment changes in pain perception was identified. ROI analysis demonstrated significant correlations in the PAG and pACC (Figure 18) but not in the amygdala. Further, activity in none of the ROIs (pACC, PAG, or amygdala) correlated with measures of locus of control or state or trait anxiety (p-values **>** 0.14).

Discussion

Here we show that uncontrollability-induced increases in pain ratings are reflected by increased activation of brain structures that are commonly implicated in pain processing. More importantly, augmented pain perception induced by uncontrollability was driven by increased activity in pACC and PAG, in line with our hypothesis. This finding extends the existing literature that had thus far not investigated brain regions responsible for uncontrollability-induced pain augmentation. Contrary to our hypothesis, the amygdala was not important for translating uncontrollability into pain facilitation: it did not show significant activation in the uncontrollable condition, and neither was its signal related to pain increase. Hence our results indicate important roles for the PAG and pACC in driving pain augmentation by uncontrollability but do not provide evidence for a role of the amygdala.

Dissociation of pain perception in controllable and uncontrollable conditions

During painful trials in the controllable condition, subjects showed temporal summation indicated by down-regulation of the temperature. Temporal summation in similar paradigms has been shown before (Becker et al., 2011; Hölzl et al., 2005; Kleinböhl et al., 1999) and has been related to NMDA receptor-mediated wind-up of spinal cord neurons (Eide, 2000; Kleinböhl et al., 2006), not necessarily involving supraspinal mechanisms. This notion is supported by our finding that temporal summation in the controllable condition was not associated with increased activation in any pain-processing area, although nociceptive stimulation in this condition resulted in typical pain-related activation. This finding also indicates that subjects successfully kept their sensation constant. Further, we observed a positive correlation between self-adjusted temperature change and the subscale “powerful others” of the IPC locus of control scale indicating that the

degree of external control belief influences the extent of temporal summation. This is in accordance with theories of learned helplessness, as subjects who tend to belief that powerful others control their lives have increased pain sensations even in controllable situations. In line with previous psychophysical work using similar paradigms (cf. Becker et al., 2011; Hölzl et al., 2005), pain perception in the uncontrollable trials was dissociated from the controllable trials. Importantly, in the uncontrollable condition, the same nociceptive inputs were re-applied, thereby accounting for peripheral and spinal sensitization. Now, these temperature profiles were consistently rated by the subjects as increasingly painful over the course of the individual trials, clearly demonstrating the pain-enhancing effects of uncontrollability (Borckardt et al., 2011; Müller, 2011).

Pain-processing areas reflect uncontrollability-induced pain augmentation

Areas that reflected the increased subjective ratings in the uncontrollable pain condition mainly receive nociceptive input via spinothalamic (Dum, Levinthal, & Strick, 2009) and spinopallidal (Braz, Nassar, Wood, & Basbaum, 2005) tracts and are typically found to be activated in pain imaging studies (Apkarian et al., 2005). These areas comprised bilateral anterior insula, rostral and mid ACC, supplementary motor cortex, bilateral thalamus, and lentiform nucleus. This is congruent with previous studies on uncontrollable pain stimuli (Mohr et al., 2005; Salomons et al., 2004) and indicates that the loss of control not only led to a reinterpretation of the painful stimulation (Wiech et al., 2006), but also to amplified processing of the nociceptive input. Nociceptive neurons that encode stimulus intensity have been reported in the insula (Ostrowsky et al., 2002), ACC (Sikes & Vogt, 1992), thalamus (Kenshalo, Giesler, Leonard, & Willis, 1980) and lentiform nucleus (Chudler & Dong, 1995), and imaging studies using increasing stimulus intensity to increase pain perception have observed that the signal in insula, ACC, supplementary motor cortex, basal ganglia, and thalamus scales linearly with the perceived intensity (Coghill et al., 1999; Loggia et al., 2012; Seminowicz & Davis, 2007). Taken together, this suggests that activation of these regions reflects increased perceived pain intensity in the present study as well as other studies on pain uncontrollability, rather than being regions driving the increases in pain perception.

Brain areas driving uncontrollability-induced pain augmentation

We identified the PAG as being an important region behind the additional sensitization in the uncontrollable condition, possibly driven by the pACC. Due to the original design of the present study that allowed capturing moment-to-moment changes of pain perception, we were able to add to previous work that had observed increased activation of these regions during uncontrollable compared to controllable painful stimulation (Mohr et al., 2005; Salomons et al., 2004; Wiech et al., 2006).

Albeit originally identified to inhibit pain, it is now clear that the descending pain modulatory system can equally facilitate spinal transmission of nociceptive information (Porreca et al., 2002). The brainstem, and in particular the PAG and the rostroventral medulla (RVM), are key relay structures of the descending pain modulatory system. The PAG integrates input from the spinal cord, hypothalamus, amygdala, pACC, insula, and orbitofrontal cortex (Basbaum & Fields, 1984; Hadjipavlou, Dunckley, Behrens, & Tracey, 2006), and can exert pro- or anti-nociception depending on the respective circumstances and cognitive-emotional state (Bingel et al., 2006; Fairhurst, Wiech, Dunckley, & Tracey, 2007; Heinricher, Martenson, & Neubert, 2004; Monhemius, Green, Roberts, & Azami, 2001; Valet et al., 2004). An elegant body of work in animals has provided evidence that different sub-regions of the PAG are associated with different coping styles in response to painful stimuli (reviewed in Lumb, 2004). The ventrolateral PAG promotes passive coping when stimuli are inescapable (Keay, Clement, Depaulis, & Bandler, 2001). Uncontrollability and inescapability are distinct but closely related concepts and it could therefore be hypothesized that it is the ventrolateral portion of the PAG that is instrumental in uncontrollability-induced pain augmentation, although the spatial resolution of human fMRI does not allow making this distinction.

The pACC projects to the PAG-RVM-spinal cord descending system (Müller-Preuss & Jurgens, 1976) and might via this route contribute to pain facilitation in the context of uncontrollability. The pACC has been suggested to play a major role in pain modulation by integrating contextual information and sensory processes (Bingel et al., 2006; Rainville, 2002). Interestingly, cognitive-emotional pain modulation by the pACC, demonstrated in the context of placebo analgesia (Bingel et al., 2006; Eippert et al., 2009) and distraction (Valet et al., 2004), has thus far only revealed pain inhibition. However, it is known from a study investigating sensitization induced by cutaneous application of capsaicin that activation in the pACC can also reflect pain facilitation (Lorenz et al., 2002). To our knowledge, the

present study is the first to present data implicating the pACC in pain facilitation by cognitive-emotional mechanisms.

Uncontrollability has been associated with a reappraisal of the meaning of and increased attention to the painful stimulus in addition to being related to increased anxiety (Arntz & Schmidt, 1989; Bowers, 1968). We did not find evidence for a contribution of the amygdala and measures of anxiety to be related to uncontrollability-induced pain augmentation. Thus, uncertainty or anxiety might not play key roles in the paradigm employed here, possibly because subjects were familiarized with the experiment in an extra session. The relatively low scores in state anxiety support this notion. This finding is exciting because it suggests that the effects of uncontrollability on pain extend beyond effects of uncertainty or anxiety.

In summary, our results indicate that loss of control leads to activation of a pro-nociceptive circuitry, involving PAG and pACC. The pACC might translate the uncontrollability into pain facilitation, executed by descending pathways from the PAG to the spinal cord, amplifying transmission of incoming nociceptive signals. This amplification would result in enhanced activation of spinothalamocortical and spinopallidal pathways, resulting in increased activation of pain processing structures as well as increased pain perception, as observed in the present study.

Clinical implications

Chronic pain is in many instances uncontrollable. Evidence shows that experiencing uncontrollable pain can lead to hyper-vigilance to pain (Aldrich, Eccleston, & Crombez, 2000), learned helplessness, and depression, resulting in a self-amplifying vicious circle (Arntz & Schmidt, 1989). Further, losing control over pain potentially increases fear of pain and interferes with task performance (Crombez et al., 2008). In contrast, perceived control has been demonstrated to decrease pain and discomfort in acute pain (Thrash et al., 1982) and is associated with better functioning in chronic pain patients (Tan, Jensen, Robinson-Whelen, Thornby, & Monga, 2002). Understanding the endogenous pain facilitatory mechanisms underlying uncontrollability-induced pain augmentation might inform clinical approaches of pain management and therapy. For example, the results of the present study suggest that addressing anxiety is not sufficient to abolish uncontrollability-induced pain augmentation and point at the usefulness of promoting coping styles and strategies aimed at increasing the patient's sense of

control, for example by active coping and reinterpretation of pain sensations (Haythornthwaite et al., 1998) or acceptance-based therapies (McCracken, 2004).

Limitations

Contrary to our hypothesis, we did not identify the amygdala as driving the uncontrollability-induced pain augmentation. However, the amygdala is a small brain structure located near air-filled cavities, which can cause reduced BOLD sensitivity due to intravoxel dephasing and susceptibility artefacts, compromising the signal-to noise-ratio (LaBar, Gitelman, Mesulam, & Parrish, 2001). In addition, the amygdala signal is often found to be transient (Bordi & LeDoux, 1992) and therefore, the sensitization occurring over relatively long periods might have precluded finding significant activation within the amygdala. For these two reasons, we cannot fully exclude that the amygdala might contribute to uncontrollability-induced pain augmentation. The aim of this study to differentiate between brain areas driving uncontrollability-induced pain augmentation and those reflecting increased pain was successfully achieved. Functional connectivity analysis might reveal further insights into the interplay between brain regions driving uncontrollability-induced pain augmentation. Due to the characteristics of the stimuli applied (tonic stimulation) functional connectivity analyses were not an option for this study but should be considered for future investigations.

4. General Discussion

The present studies investigated the effects of nocebo-conditioning and uncontrollability, two psychological factors capable of facilitating the pain sensation. The results show that after changing the behavioral context in either case, physically identical stimulus intensities were perceived as more painful than before the manipulation. The pain modulatory effects were assessed on different response channels, resulting in notably increased subjective ratings of the pain sensation, altered implicit-behavioral responses, as well as activity in brain regions associated with pain processing. In detail, contingency awareness was not a necessary condition for nocebo conditioning, but successfully conditioned subjects were more sensitive in response to heat-pain than non-successfully conditioned subjects. Subjective pain enhancement in an uncontrollable task was reflected in increased brain activity in pain processing areas, modulated by pACC and PAG but not amygdala. The studies have important clinical implications as they contribute to a better understanding of pain facilitatory mechanisms and can potentially improve treatment options.

4.1 Classical Conditioning of the Nocebo Effect

Two different experiments demonstrated that a nocebo effect in heat-pain perception could be induced by means of classical conditioning without giving additional verbal suggestions or employing a cue that is prone to raise expectations from the outset (cf. Jensen et al., 2012). Previously, it was suggested that expectation, but not conditioning, is the crucial mechanism leading to nocebo-hyperalgesia, in contrast to placebo-hypoalgesia, in which both conditioning and expectation were assumed to play a significant role (Colloca et al., 2008; Petrovic, 2008). However, most studies did not test effects caused by conditioning separately (Benedetti et al., 2003; Benedetti et al., 2006; Bingel et al., 2011; Colloca & Benedetti, 2006; Colloca et al., 2008; Voudouris et al., 1985; Voudouris et al., 1989). Thus, our results emphasize the important role of learning mechanisms in the development of the nocebo effect. Unquestionably, a very common route to develop nocebo-hyperalgesia is the generation of an explicit expectation of pain increase (Colloca et al., 2009; Schmid et al., 2013). However,

when a nocebo effect is induced by means of conditioning, it possesses characteristics of a conditioned response, which distinguishes it from an effect that is driven by an explicit expectation. It can be suggested that conditioned effects are more stable than effects caused by expectations, which has already been shown for placebo-hypoalgesia in patients with atopic dermatitis (Klinger et al., 2007). Further, conditioned responses pertain characteristics like dose-dependency (Pihl & Altman, 1971; Ross & Schnitzer, 1963), effects of the reinforcement schedule, extinction (Knowles, 1963), and prediction of the magnitude of the CR from the acquisition phase (Jensen et al., 2012). Knowledge on the qualities of the nocebo response is important because it leads to a better understanding of the underlying mechanisms; it becomes relevant when a nocebo effect is implemented in an experimental context as well as prevented or eliminated in the clinical context.

As expected, not all subjects showed a nocebo effect, i.e., were successfully conditioned. In Study 1, 71.4 % and in Study 2, 50 % of the subjects were considered responders. We employed a responder criterion that depended on the difference between ratings of the stimulus presented after CS+ and CS– in the test phase of the experiment. This allocation was applied to the implicit-behavioral and autonomic measure (the latter only in Study 2), avoiding circular reasoning (Kriegeskorte et al., 2009). Beforehand, we investigated potential confounding variables that derived from a priori differences between subjects and difference during the learning phase. However, a classification of the subjects into learners and non-learners was not successful upon these analyses. For the placebo effect, usually a typical responder rate of 35 % to 66 % is assumed (Beecher, 1955; Rief, Hofmann, & Nestoriuc, 2008; Wickramasekera, 1980). No independent estimates for the nocebo effects have been published yet and there are no studies on separate responder rates after experimental manipulations by verbal suggestions or by conditioning. One study found that 19 % of the healthy subjects participating in a drug trial reported side effects despite being in the placebo group (Rosenzweig, Brohier, & Zipfel, 1993). Further, according to a focused review (Barsky et al., 2002), approximately 25 % of patients receiving placebos report adverse side effects. These findings indicate that the responder rates found in the present nocebo studies were presumably on average (Study 2) or above average (Study 1).

A number of studies tried to establish personality traits that identify subjects that are prone to respond to placebo manipulations (e.g., hypnotizability, Wickramasekera, 1980; dispositional optimism, Morton et al., 2009; reward-

related personality traits, Schweinhardt et al., 2009). But only few studies investigated correlations of personality traits with the nocebo effect. The results of Study 2 suggest that motivational style (assessing reward-related personality traits) and suggestibility were not related to the nocebo effect, further emphasizing that the nocebo effect is not just a reversed placebo effect. There is some evidence that neuroticism (Davis, Ralevski, Kennedy, & Neitzert, 1995), anxiety (Andrykowski & Redd, 1987; Uhlenhuth et al., 1998), pessimism (Geers, Helfer, Kosbab, Weiland, & Landry, 2005), hypochondriacal fear (Davis et al., 1995), and somatization (Papakostas et al., 2004; Uhlenhuth et al., 1998) are positively related to the tendency to experience adverse side effects. A recent study in the context of nocebo-hyperalgesia showed that pain catastrophizing – and here especially the helplessness subscale – correlated with the nocebo effect (Vogtle et al., 2013). Another study showed that after a short (comprising five trials for each condition), but not a long conditioning procedure (comprising 20 trials for each condition), nocebo responses were positively correlated with state and trait anxiety when using painful stimuli and negatively correlated when non-painful stimuli were employed (Colloca et al., 2010). The authors concluded that by a long conditioning procedure, dispositional factors might become overruled. Further, increased anxiety accompanying a nocebo effect after an expectation/conditioning manipulation was reported by Bingel et al. (2011). These results support the notion that anxiety-triggered CCK activation might cause nocebo-hyperalgesia (Benedetti et al., 1997; Ploghaus et al., 2001). However, in another study that induced nocebo-hyperalgesia in a visceral pain model by expectancy manipulation, no correlation of the nocebo effect with state anxiety was reported (Schmid et al., 2013). One explanation by the authors was that with an expectation manipulation, a “cognitive pain modulation” might have been triggered compared to a conditioning manipulation that might rather trigger “emotional pain modulation” so that no changes in negative emotions could be detected. However, in a recent study that induced nocebo-hyperalgesia by an observational learning paradigm no correlation with pain-related fear (as assessed with PASS) was found as well (Vogtle et al., 2013). Accordingly, in the present Study 1 none of the implemented anxiety measures (STAI, PASS, FPQ) was related to the nocebo effect, which might be due to the relatively large number of trials (15 trials for each condition) comparable to the long conditioning procedure employed by Colloca et al. (2010). In Study 2, unexpectedly, a negative correlation of the hyperalgesic nocebo response with trait anxiety appeared. In concluding, the conflicting results of the present and previous studies suggest that the role of

anxiety in the context of the nocebo effect remains to be elucidated and needs further investigation.

Our results rather suggest that pain sensitivity might play a role in the responsiveness for nocebo conditioning: In Study 1, in which the employed stimulus temperatures of the US depended on the pain threshold, successfully conditioned subjects had significantly higher pain thresholds than not successfully conditioned subjects. In Study 2, the stimulus temperatures were determined by a subjective rating procedure and during conditioning, successfully conditioned subjects reported significantly higher subjective ratings of the painful stimulus (US) compared to subjects that were not successfully conditioned, although stimulus intensities were comparable. These observations both point in the same direction and indicate that subjects that (subjectively or objectively) perceive more nociceptive input are more likely to develop a nocebo response. Further, Study 2 showed that autonomic responses were different between non-learners and learners, the latter having increased heart rate and decreased heart rate variability parameters. Evidence shows that within the first three seconds of painful stimulation, the heart rate is determined by the stimulus intensity but after approximately six seconds it is rather related to the subjective evaluation of the stimulus (Möltner et al., 1990). Our results on the autonomic measures therefore might reflect the increased pain ratings of the learners. Accordingly, reduced increases in heart rate and sympathetic responses (0.15 Hz peak in spectral analysis of HRV) along with decreased pain ratings were observed in two studies on placebo analgesia induced by expectation manipulation and sham drug treatment (Pollo et al., 2003). Taken together, the results indicate that placebo-hypoalgesia is accompanied by reduced heart rate and sympathetic activity, whereas nocebo-hyperalgesia seems to be accompanied by increased heart rate and decreased parasympathetic activity.

With the subjective (difference between VAS_2 and VAS_1) and behavioral measure (difference between end and initial temperature of the self-regulation procedure), the short time development of the conditioned nocebo response was investigated for the first time. We interpreted the decreasing/decreased sensitivity in the absence of the nocebo cue as decomposition of the the conditioned response over time (“decay”). This decay was observed in Study 1: Both the subjective as well as the behavioral measure indicated increased adaption following the subjective nocebo response. The short time development of the nocebo response is potentially clinically relevant, as it might have direct behavioral consequences (e.g., by leading to relieving postures). However, in Study 2 these outcomes were

not replicated, which might be due to the modifications in Study 2 that generally led to a weaker conditioning effect compared to Study 1.

The procedures employed in both nocebo studies were very similar. Both the CS and the US were temperature stimuli, i.e., processed within one system. Further, in contrast to implementing an association between a painful temperature and an artificial or irrelevant CS (i.e., visual cues, like a cross and a circle), having one temperature signaling another, higher temperature, harnesses naturally occurring relations (Domjan, 2005). Although the CS was not directly fear-relevant, we assume that preparedness (Seligman, 1972) facilitated the coupling of two temperature stimuli. However, in Study 2 we made some modifications in order to gain more information on specific determinants of the conditioned nocebo effect. One aim of Study 2 was to replicate the results of Study 1 in the subjectively painful range. We therefore employed a different strategy to determine the stimulus temperatures. Instead of using the individual pain threshold and units of JNDs as a basis from which the non-painful (pain threshold – 2.2 °C) and painful (pain threshold + 1.5 °C) stimuli were determined, we adapted a procedure that was based on the subjective evaluation of different intensities (Price et al., 1999). Different stimulus temperatures were presented to the subject and with a robust linear regression we predicted stimulus intensities that matched an individual rating of 50 and 70. Thereby we could increase the subjectively perceived level of stimulus intensity compared to Study 1. However, in the learning phase, the moderately painful stimulus, which was supposed to correspond to a rating of 50, was evaluated as non-painful. But then again, in the test phase, when cued by CS–, the successfully conditioned subjects rated this stimulus as indicated (M = 52), while the non-successfully conditioned subjects rated it as just painful on average (M = 40.5). When cued with CS+, the successfully conditioned subjects perceived the stimulus as clearly painful but the subjects who were not successfully conditioned rated it as non-painful. In the test phase of Study 1, the temperature was evaluated as non-painful after either cue. Interestingly, in the test phase of both studies, the successfully conditioned subjects rated the applied stimuli as significantly more painful than the non-successfully conditioned subjects on average. This indicates that, in a given context, the more intense stimuli are perceived, the more likely a nocebo effect develops. Considering the function of pain to protect the body from harm, this mechanism seems reasonable, as the motivation to end the painful condition or escape from it is increased by the nocebo effect.

In order to increase differentiation, we used a temperature pulse (quick increase of the temperature from baseline directly followed by a quick decrease back to baseline before presentation of the US temperature) instead of static temperature stimuli as CS, since the results of Study 1 showed that CS differentiation was beneficial for the nocebo effect. As a consequence, a trace conditioning paradigm was implemented as compared to Study 1, in which the conditioning paradigm was a borderline case of delay and trace conditioning. Further, to strengthen the nocebo effect, we prolonged the test phase by two trials and introduced three booster trials in the test phase of Study 2, in which we presented the painful stimulus cued by CS+. However, this modification might have corrupted the conditioning effect due to changes in adaptation level (Helson, 1947) and explain that less subjects were successfully conditioned.

In summary, the presented studies show that, other than suggested (Benedetti et al., 2003; Colloca et al., 2008; Petrovic, 2008), classical conditioning is a feasible method for inducing a nocebo effect in the context of pain. Some of the modifications in Study 2 presumably led to an attenuation of the conditioning effect. The nocebo response was assessed on different measures, indicating that it is a real psychobiological phenomenon, not just a result of decreased pain being reported.

4.2 The Role of Awareness in Nocebo-Conditioning

The two present nocebo studies show that a conditioned nocebo effect can emerge without contingency awareness. In both studies, two thirds of the subjects were unaware of the contingency between the nocebo cue and the following painful stimulation. Expectancies are a common epiphenomenon of conditioning (Stewart-Williams & Podd, 2004), but subjects who remain contingency unaware, by definition, cannot develop an explicit expectation in regards to the nocebo cue. Thus, this result emphasizes the finding that a nocebo effect in pain perception does not depend on explicit expectation and can be induced by conditioning exclusively. This outcome has far reaching consequences for the clinical context, as it suggests that patients, unbeknownst, might learn associations between various cues and subsequent pain increase without even being able to recognize what exactly has caused the worsening. However, this prevents a patient from developing pain control strategies and can thereby contribute to the chronification or maintenance of a pain condition.

Previously, a study by Benedetti et al. (2003) showed that a negative verbal suggestion reduces markedly previous pharmacological placebo conditioning with an analgesic drug (keterolac). On the contrary, verbal suggestions of cortisol increase and growth hormone decrease did not lead to the according changes in hormone levels, and the same verbal suggestions after conditioning with sumatriptan (causing increase in growth hormone and decrease in cortisol) did not alter the conditioned effects (i.e., increase in growth hormone and decrease in cortisol; Benedetti et al., 2003). Based on these observations, it was suggested that for conscious physiological processes (like pain or motor performance), not conditioning but expectation (possibly formed by conditioning) is the crucial mechanism leading to a placebo or nocebo effect. Unconscious physiological processes, like hormone secretion, were sought to be mediated by conditioning only, as evidence showed that conditioning, but not verbal suggestions, affected the plasma concentration of growth hormone or cortisol (Benedetti et al., 2003). Likewise, it was shown that respiratory depression, an adverse side effect in analgesic therapy with opioids, occurs after conditioning with the partial opioid agonist buprenorphin (Benedetti et al., 1998; Benedetti et al., 1999). This effect was unnoticed by the subjects and did not depend on the hypoalgesic placebo effect so that the authors concluded that it was caused by conditioning and not by expectation. The fact that, in the present studies, contingency unaware subjects developed a nocebo effect strongly indicates that conditioned nocebo effects are not limited to unconscious physiological processes, as hypothesized in the past. Our results even go one step further than a recent study by Jensen et al. (2012), in which was shown that a nocebo effect can be activated by subliminally presented CS after an explicit conditioning procedure (using supraliminally presented CS) had taken place (although contingency awareness was not assessed here). Whereas this study suggests that explicit expectations are not necessary to *activate* nocebo-hyperalgesia, the present results amend this by showing that explicit expectations are not necessary in the *induction* of the nocebo effect, either. Only one other study directly tried to induce placebo-hypoalgesia by implicit conditioning (Martin-Pichora et al., 2011). In the implicit condition, an inert “anesthetic” and a control cream were applied on healthy subjects’ forearms and a tactile cue (upward or downward strokes to apply the cream) was used as CS and coupled to a lowered temperature during conditioning. In the test trial, the subjects did not know which cream was applied and only the direction of the strokes served as a cue. The results show that pain ratings were not reduced after administration of the placebo cue, indicating that the implicit conditioning procedure was not

successful. However, only one subject reported being aware of the different directions in which the cream was applied. Thus, the placebo effect might have been prevented by the lack in CS differentiation. Apart from a number of procedural concerns related to this study, the application of a cream seems disadvantageous in this specific context as this might already induce explicit expectations and cause placebo effects that are not separable from effects caused by the implicit conditioning.

Implicit conditioning, beyond the placebo and nocebo context, is discussed controversially (Clark et al., 2002; Dawson & Biferno, 1973; Lovibond, 2002; Manns et al., 2002). Whereas some authors reject the possibility of conditioning without contingency awareness (e.g., Dawson & Schell, 1985; Dawson, 1970; Dawson & Biferno, 1973), others assert that it can only occur under specific conditions (e.g., autonomic conditioning, Knight, Nguyen, & Bandettini, 2003; Manns et al., 2001; Perruchet, 1985; Schultz & Helmstetter, 2010; with fear-relevant CS and aversive US, Esteves, Parra, Dimberg, & Öhman, 1994; Öhman, 1986; Soares & Öhman, 1993; evaluative conditioning, Stevenson, Prescott, & Boakes, 1995; Stevenson, Boakes, & Prescott, 1998). Imaging studies support a dual process model of conditioning, assuming a double dissociation of contingency awareness and conditional fear responses. Whereas the former is sought to depend on involvement of hippocampal structures, the latter presumably rely largely on processing in the amygdala (Bechara et al., 1995; Knight, Waters, & Bandettini, 2009; Tabbert et al., 2011). Further, implicit operant learning of pain sensitivity has been demonstrated in healthy subjects as well as fibromyalgia patients using similar response measures as in the present studies (Becker et al., 2012; Hölzl et al., 2005).

A limiting factor in both nocebo studies was the weak test of contingency awareness. The subjects were interviewed after the experiment and answered questions regarding the sequence of the temperature stimuli (i.e., CS and US) with the aid of a flowchart depicting the temperature course of a trial. This approach has several pitfalls, for example by the time of the interview, subjects could already have forgotten about the contingencies (Lovibond, 2002). A superior alternative would have been a concurrent rating of US expectancy; however, this most certainly would have increased awareness.

To sum up, in both studies, contingency awareness was no necessary condition for the development of a nocebo effect. This indicates that conditioning without expectancy is sufficient to induce a nocebo effect, even in the (partly) conscious

physiological process of pain (Benedetti et al., 2003). This result has important implications for the clinical context (please refer to section 4.5).

4.3 Increased Sensitization Induced by Uncontrollability

As a second pain facilitatory psychological factor, the effect of uncontrollability on perceived pain was investigated. Study 3 shows that when changing an experimental task from being controllable to uncontrollable, the same nociceptive input, previously self-adjusted to feel constant, is evaluated as getting increasingly painful. During the controllable task, i.e., when controlling the stimulus temperature and keeping it constant, subjects sensitized during painful trials (regulated the temperature down) and habituated during non-painful trials (regulated the temperature up). When, during the uncontrollable task, the same nociceptive input that was self-adjusted to feel constant was replayed, subjects rated it as getting more and more painful. This additional pain enhancement was reflected in increased activity in pain processing brain areas, like bilateral insula, rostral and mid ACC, supplementary motor cortex (BA8), bilateral thalamus, and lentiform nucleus. Further, brain areas known to play a role in pain modulation, namely PAG and pACC, appeared to drive this additional sensitization.

Previous evidence shows that having a sense of control leads to decreased pain sensitivity (Borckardt et al., 2011; Bowers, 1968; Litt, 1988; Weisenberg et al., 1985) and, on the other side, having no control increases the pain sensitivity (Crombez et al., 2008; Müller, 2012; Staub et al., 1971; Wiech et al., 2006). Different theories try to explain the pain increase induced by uncontrollability. Anxiety (Bowers, 1968) is often observed in this context (Crombez et al., 2008), but rather constitutes an epiphenomenon than an explanation for the pain increase (Arntz & Schmidt, 1989). In the present experiment, however, state and trait anxiety were not related to the extent of pain increase caused by uncontrollability, possibly because the subjects were familiarized with the experimental procedure before the actual testing. Further, the amygdala is well known for its involvement in fear-related processes (Neugebauer et al., 2004; Phelps & LeDoux, 2005) and we did not observe increased activation here (although this might be due to methodological difficulties in imaging this brain region). A widely accepted explanation for controllability-related changes in pain sensation refers to a reappraisal of the painful stimulation. According to Arntz & Schmidt (1989), (perceived) control alters the meaning of painful stimulation by reducing its

perceived threat. The same nociceptive input that was self-adjusted to feel constant during the controllable task presumably was reinterpreted to feel painful after losing *instrumental control* because the subject could no longer oversee the extend of the pain and potential harm (*minimax hypothesis*; Miller, 1979). Along these lines, in the present study, the sensitization during controllable painful stimulation positively correlated with an external locus of control belief ("powerful others"), indicating that locus of control beliefs can enhance the pain perception even when instrumental control is exerted. Appositely, evidence shows increased activity in ventrolateral PFC, a brain area known to be involved in reappraisal processes, in controllable compared to uncontrollable conditions (Wiech et al., 2006), (Kalisch, Wiech, Critchley, & Dolan, 2006; Ochsner & Gross, 2005). Similarly, animal studies suggest a role for ventral medial PFC and it is hypothesized that this brain region inhibits activity in the doral raphe nucleus in rats when stress is controllable (Amat et al., 2005). However, in the present study, no significant prefrontal activity was detected. Correspondingly, we also did not observe behavioral immunization (Seligman & Maier, 1967), i.e., an effect in which experience of control leads to resilience in later situations of no control (Amat, Paul, Zarza, Watkins, & Maier, 2006) and which seems to rely on ventral medial PFC as well.

The results show that the pain increase that was induced by uncontrollability was driven by increased activity in perigenual ACC and PAG. These brain structures are well known for their role in pain modulation (Bingel et al., 2006; Fairhurst, Wiech, Dunckley, & Tracey, 2007; Heinricher, Martenson, & Neubert, 2004; Monhemius, Green, Roberts, & Azami, 2001; Valet et al., 2004), for example from studies that investigated placebo analgesia (Bingel et al., 2006; Eippert et al., 2009) and distraction (Valet et al., 2004). Accordingly, evidence shows that functional connectivity between those brain regions is high (Kong, Tu, Zyloney, & Su, 2010). Although some evidence demonstrates that pain facilitation is mediated by this circuitry of brain areas, as well (Porreca et al., 2002), previous studies almost exclusively investigated pain modulation by means of inhibition. Thus, our study contributes to a better understanding of the interrelation between pain inhibition and pain facilitation.

According to the typology of control (please refer to 1.2) previous studies mainly used procedures that classify as *self-administration* and/or *instrumental control*. Most studies employed manipulations, in which the subject could self-administer or end a painful stimulation in the controllable condition and was administered a yoked painful stimulation by the experimenter in the uncontrollable condition. For

instance, in the controllable condition, the subjects pulled a rope to apply the painful stimulation after a verbal command (and sometimes were able to end the stimulation by pulling again; Mohr et al., 2008) and in the uncontrollable condition the experimenter applied the stimulation after a variable time interval following a verbal signal (Helmchen et al., 2006; Mohr et al., 2005) A similar procedure was used by Müller (2011; Müller, 2012); here the subjects could self-administer electric shocks within a time window of 10 seconds, contrasted to administration of the shocks by the experimenter according to a random schedule. In a study by Wiech et al. (2006), the subjects were told that they could stop painful stimuli when the pain becomes unbearable in the self-controllable condition, whereas the painful stimulation was stopped by experimenter or a computer in the externally controlled condition. Another possibility to manipulate perceptions of control was posed by subjects' performance in reaction time tasks. Subjects were led to belief that they could reduce the duration of a painful stimulation if they responded correctly and quickly enough in the controllable condition, but their response had no consequence in a non-controllable condition (controllability or lacking control was prompted before each trial; Borckardt et al., 2011; Salomons et al., 2004). In the present procedure, contrasting to previous studies, subjects had direct control over the intensity of the nociceptive input (i.e., the applied temperature) during the controllable trial. This approach seems to be a much more powerful manipulation of perceived control than a decision at which point in time a painful stimulus is self-administered within a narrow time frame. It further allowed tracking the changes in pain sensation over time in both the controllable and uncontrollable conditions. This made it possible to identify not only brain regions that correlated with the pain increase but also those regions that drove the pain augmentation.

Some limitations deserve mention concerning the study on uncontrollability. Due to the yoked-control design, controllable trials always preceded uncontrollable trials in the two functional scans that were separated by the anatomical scan. Therefore, an effect of order cannot completely be ruled out, although we verified that pain ratings across trials did not change (cf. Figure SIV), suggesting that the increase in pain perception in the uncontrollable trials was not due to sensitization over time. Another aspect concerns the characteristics of the experimental tasks. The controllable and the uncontrollable tasks were not completely equivalent. In the former, the subjects had to actively keep a temperature constant whereas in the latter, they had to passively rate the temperature throughout the trial. An 'active task', however (e.g., in which subjects have to control something else but

pain), would prevent the assessment of perceived pain intensity and therefore preclude ensuring that stimulus uncontrollability in the task indeed increased pain perception. In our view, it is important to not only show more brain activation in pain processing regions to infer successful pain augmentation by uncontrollability, but to also show the effect on a behavioral level. Therefore, it was necessary to assess the perceived pain intensity. Furthermore, the uncontrollable task allowed to observe continuous changes in perception (while equating motor output between the two tasks), thereby differentiating between brain regions that reflected and those that drove increased pain perception. Also, it could be argued that an 'active' component is an inherent constituent of exerted controllability. In line with this view, controllable conditions in previous studies on pain controllability typically contained an active component that was absent in the uncontrollable condition (e.g., termination of painful stimulus by the subject vs. by another person). Further, having control over something else while simultaneously receiving pain stimuli would introduce other important confounds, most importantly distraction, which is known to decrease pain perception (Valet et al., 2004; Villemure et al., 2003). A second limitation regarding the experimental tasks is related to the different response channels represented by the behavioral task and the subjective rating. As already discussed (please refer to sections 1.3 and 4.4), pain is a multidimensional phenomenon and different measures do not have to correspond. It is thus possible that the pain increase that was observed in the subjective ratings during the uncontrollable trials was (partly) due to the method of assessment and level of processing (i.e., implicit-behavioral versus subjective-verbal). In conclusion, we think that the advantages of our experimental paradigm outweigh potential disadvantages; however future studies should incorporate different methods of assessment within both the controllable and the uncontrollable task in order to exclude potential confounding sources.

In summary, in the present study on uncontrollability, increases in pain report after induction of a nocebo effect led to increased activity in pain processing regions as well as involvement of the descending pain modulatory circuit, including rACC and PAG, which were shown to drive the changes in pain sensation induced by uncontrollability.

4.4 Multidimensional Assessment of the Pain Response

Pain is a multidimensional experience that can be assessed on different response channels. Most studies on pain ask the subjects to give a verbal rating of the pain sensation and/or pain unpleasantness (VAS, NRS). But verbal ratings are prone to response bias and demand characteristics (Kienle & Kiene, 1997; Hrobjartsson & Gotzsche, 2001; Chapman et al., 1985). Further, they only represent one facet of the pain experience and here only aspects that are explicitly represented. The subjective evaluation of a painful event certainly is of major interest in research as well as in clinical routine, but it is not sufficient to represent the complex experience of pain as a whole. In order to get a more comprehensive picture of the pain experience, in the presented studies, we additionally assessed a dynamic behavioral measure indicating the change in pain sensation over a short period of time, thereby observing adaptation effects on an implicit, behavioral level. Concretely, the subjects had the task to keep the temperature sensation constant by operating a computer mouse. The self-adjusted change in temperature thus is interpreted as temporal summation (self-adjusted decrease of the temperature) or adaption (self-adjusted increase in temperature). It can be assumed that this behavioral measure is an implicit indicator of the pain sensitivity as the subjects are not aware that their temperature adjustments constitute a type of response measure for their subjective perception (Becker, 2009; Hölzl et al., 2005). In addition to the implicit-behavioral response, autonomic measures (heart rate and HRV) as correlates of the pain perception were assessed. Autonomic variables can shed light on the affective-motivational component of the pain experience and also have the advantage of being independent from verbal report (Möltner et al., 1990). The importance of autonomic measures in the context of pain gets obvious when referring to pain conditions that demonstrably show an involvement of the autonomic system. For instance, pain in the complex regional pain syndrome (CRPS) is at least in part thought of as being maintained sympathetically (Stanton-Hicks et al., 1995), which led to the development of interventions targeting the sympathetic nervous system (e.g., ganglion stellate blocks; van Eijs et al., 2012). In the present nocebo study (Study 2), learners and non-learners differed in their heart rate and HRV, suggesting that the autonomic response might be an indicator of successful learning. Finally, neural correlates of the pain experience were investigated. According to Chapman et al. (1985), correlates of pain can help confirming the validity of pain experiments, increase statistical power by providing additional information for hypothesis testing, and help to assess related aspects of

the pain experience, like anxiety. However, in addition to that, by understanding the neural activity that occurs during experience of a painful event, aberrant processes in pain conditions can be better explained and therapies might be tailored accordingly (for example in phantom pain, please refer to section 1). Further, the investigation of neural correlates of pain, in the future, might reveal an opportunity for diagnosis and treatment of subjects who are not communicative, for example due to advanced dementia or in states with altered consciousness (e.g., minimally conscious; Schnakers, Faymonville, & Laureys, 2009). Dissociations between different measures (Becker et al., 2011; Hölzl et al., 2005) can arise because pain is not a uniform construct. Thus, the investigation of the pain experience on different response channels is worthwhile because disadvantages of single assessment methods can be compensated and more information gathered.

The behavioral and subjective measures were adopted differentially in the nocebo studies and the study on uncontrollability. In the former, the behavioral measure indicated the decay of the conditioned response as assessed with subjective ratings within one trial and it was complemented by the subjective indicator of decay. Thereby, it was possible to show that the nocebo effect neither solely depended on demand characteristics nor was a consequence of response bias because a conditioned effect was observable in both the subjective ratings and the behavioral measure (at least in Study 1). Further, this approach allowed to evaluate the development of the nocebo response over time and characterize its decomposition. In the study on uncontrollability, the behavioral task formed the controllable condition, taking advantage of the fact that by keeping the temperature constant, the subjects exerted control over the nociceptive input. The self-adjusted temperature course was replayed for the uncontrollable condition, in which the subjects continuously rated their sensation, without having control over the nociceptive input any longer. Increases in pain sensation did not depend on peripheral sensitization because this was already accounted for during the behavioral task. Thus subjectively perceived pain increases could be attributed to the loss of control. Although, a limitation of this approach is that the experimental conditions (controllable and uncontrollable) were not completely similar, controllability was manipulated indirectly, intrinsic to the experimental tasks. This prevented the subjects from becoming aware of the purpose of the experiment and hence avoided effects of demand characteristics.

When it comes to imaging studies, the investigation of pain facilitation (Gebhart, 2004; Lorenz et al., 2005; Ploghaus et al., 2001; Suzuki, Rygh, & Dickenson, 2004; Yang & Symonds, 2012) compared to pain inhibition (e.g., distraction, Bantick et al., 2002; Tracey et al., 2002; hypnotic suggestions, Rainville, Carrier, Hofbauer, Bushnell, & Duncan, 1999; placebo effects, Petrovic et al., 2002; Wager et al., 2004) is largely neglected as well. Interestingly, neural correlates of the nocebo effect show an involvement of similar brain areas as observed in the present study on controllability. After an expectation/conditioning nocebo manipulation that led to increased intensity ratings, (Kong et al., 2008) found increased activation of the medial pain system responsible for affective-emotional and cognitive aspects of the pain perception (including ACC, insula, superior temporal gyrus, operculum, and PFC). According to Bingel et al. (2011), the analgesic effect of the opioid remifentanyl on heat pain was completely abolished after an expectation/conditioning manipulation. Further, increased pain and anxiety were mirrored in increased activation of pain processing brain regions (medial cingulate cortex, thalamus, SI, insula). Similarly, nocebo suggestions in a visceral pain model led to increased subjective pain as well as insular activation (Schmid et al., 2013). Some evidence suggests a specific involvement of hippocampal structures in the nocebo effect (Bingel et al., 2011; Kong et al., 2008), a brain area also known to play a role in learning (cf. Olsson & Phelps, 2007; Ploghaus et al., 2001). According to a review by Tracey (2010), pain modulation due to placebo and nocebo manipulations is mediated by the descending pain modulatory system, consisting of rostral ACC, hypothalamus, amygdala, PAG, and rostral ventral medulla. Although evidence is still limited in the placebo context, the involvement of these brain regions was demonstrated in several studies (Eippert et al., 2009; Petrovic et al., 2002; Wager et al., 2004; Wager, Scott, & Zubieta, 2007). Further it was shown that this descending pain modulatory system is activated in pain facilitation as well (Gebhart, 2004; Suzuki et al., 2004).

4.5 Clinical Relevance

The results of the present studies help us to understand the mechanisms underlying pain facilitation, which are largely under-investigated. The same processes might be clinically relevant. Patients “always expect the worst” as they oftentimes have not made many good experiences and feel out of control. It is known by now that the choice of the wording in doctor-patient interactions has an

impact on the patient's wellbeing (Benedetti, 2002; Lang et al., 2005). The present nocebo studies, however, emphasize the possible influence of learning processes in this context. For instance, due to latent inhibition, it is assumed that yearlong experience of ineffective therapies, which is a typical experience of many chronic pain patients, has a negative impact on later interventions (Bingel et al., 2011; Klinger et al., 2007; Voudouris et al., 1985). This effect might further be enhanced by the patient's growing sense of uncontrollability in the face of his pain condition, which leads to feelings of helplessness, fueling a vicious circle of increasing pain (Arntz & Schmidt, 1989), as evidence shows that susceptibility for a nocebo response is positively related to the extent of helplessness a person feels (Vogtle et al., 2013). The finding that a nocebo effect can develop even without the patient being aware of the contingency between cue and pain increase has especially important clinical implications. Implicit learning makes it even harder for the patient to interrupt the vicious circle of classically conditioned pain increase, operantly reinforced pain behavior (Flor, Birbaumer, & Turk, 1990) and growing sense of uncontrollability, potentially resulting in a gradual increase in pain sensitivity because the patient is not aware of the cause of the pain increase and thus cannot counteract it.

The insights of both studies could be used to improve often unsuccessful pain therapy. The choice of an intervention or medication should be carefully considered to prevent the patient from experiencing a therapy as ineffective and his pain as uncontrollable. Accordingly, when prescribing analgesic medication, the dosage should not be chosen too reluctant as reservation might be harmful. A serious potential consequence of nocebo effects (in terms of adverse side effects) is non-compliance and/or discontinuation of pharmaceutical interventions (Rief et al., 2008), which contributes to therapy failure in the end. Patients with negative prior experience as well as negative control beliefs thus need special interventions to attenuate possible adverse effects (Rief et al., 2008), for example by employing strategies to enhance cognitive re-appraisal processes. Awareness needs to be raised in patients as well as physicians and other healthcare professions. Also, the patient's coping style and locus of control beliefs need to be addressed appropriately and patients should be provided with as much control as possible. It might be worthwhile here to incorporate response channels that are oftentimes neglected, for example, biofeedback training of autonomic or neurophysiological responses (Miltner, Larbig, & Braun, 1988).

Knowledge on neural processes of pain facilitation will potentially help to develop treatment options. For example, fast repetitive transcranial magnetic stimulation

(rTMS) of the dorsolateral PFC (Borckardt et al., 2011) has shown to suppress hypoalgesic effects of controllability. Similarly, low-frequency rTMS on dorsolateral PFC blocked expectation-induced placebo-hypoalgesia (Krummenacher, Candia, Folkers, Schedlowski, & Schonbachler, 2010). As rTMS can have inhibiting or enhancing effects depending on the applied frequency, it might provide an option for pain management when the relationship between neural and psychological factors that cause or maintain a pain condition is better understood.

4.6 Conclusions and Outlook

Evidence shows that pain facilitation compared to pain inhibition is considerably understudied, despite direct relevance for the clinical context. Investigating the effects of nocebo-conditioning and the induction of uncontrollability, complex response patterns were observable in subjective and implicit behavioral response channels as well as autonomic and neural correlates. The results show that psychological factors exert powerful influence on the pain experience although many aspects need further clarification. In addition to that, a multidimensional assessment of the pain perception and its correlates proves beneficial, providing several starting points for the future assessment of pain facilitation:

- The present results show that nocebo-conditioning and uncontrollability can increase the pain sensitivity in healthy volunteers. It is yet plausible that the nocebo effect or classical conditioning in general as well as feelings of uncontrollability may play a role in the development and maintenance of chronic pain. The present paradigms could be employed on patients to further explore this hypothesis.

- A number of alterations in the experimental procedure presumably attenuated the conditioned nocebo effect in Study 2. Future studies should optimize the conditioning procedure by systematically investigating determinants of successful conditioning (e.g., conditioning design).

- The present nocebo studies were the first to consider the temporal course (i.e., decay) of the nocebo responses. Similarly, consequences of uncontrollability have never been studied before under the aspect of development over time. Further, implicit-behavioral indices of pain processing are rarely implemented. These approaches should be pursued

and patients with pain conditions should be investigated because temporal characteristics and implicit-behavioral indices of pain processing are potentially relevant in a clinical context.

- Nocebo-conditioning and uncontrollability paradigms might complement each other in order to further explore a potential common ground of neural correlates of pain facilitatory effects.

- Anxiety is hypothesized to play a role both in the nocebo effect and in uncontrollability, however, experimental support is inconsistent. Future studies should clarify this issue, for example by implementing repeated interrogation on the current level of anxiety.

- Uncontrollability and, as a consequence, learned helplessness are relevant not only in chronic pain, but also in depression and posttraumatic stress disorder (Başoğlu & Mineka, 1992). Accordingly, the present paradigm could be modified and applied to these patient populations in order to further elucidate the mechanisms at work.

Hence, the plans for future applications and development of the present results are threefold: (1) systematic investigation of experimental aspects (e.g., conditioning design) that determine the success of the nocebo-conditioning procedure (e.g., stability, effect size); (2) the present paradigms should be applied to patient groups (e.g., chronic pain, depression, etc.) in order to elucidate underlying mechanisms of the respective pathological condition; (3) the present paradigms can be utilized to investigate common neural correlates of pain facilitatory effects.

Summary

Nocebo effects and uncontrollability are important psychological factors in pain facilitation and play a major role the context of acute and chronic pain. However, the precise mechanisms in both phenomena that lead to pain increase remain understudied. The general aim of the three studies contained in this thesis was to shed light on mechanisms of conditioning-induced nocebo effects and neuronal processes during uncontrollability-induced pain increase. For this purpose, experimental designs were employed that assessed the pain perception and its epiphenomena on multiple response channels (subjective verbal report, behavioral response, autonomic response, neuronal activity).

In the first study, a conditioning procedure was developed without additional verbal suggestions or employment of cues that are prone to induce expectations of pain relief or worsening. The results indicated that conditioning can induce a subjective nocebo effect, even when subjects are contingency unaware (implicit conditioning). The decay of this conditioned response over time was observable in subjective as well as behavioral measures. Neither state nor trait anxiety or measures of anxiety specifically related to pain showed a correlation with this nocebo effect in the subjectively non-painful range.

The second study adapted the conditioning procedure in order to induce nocebo-hyperalgesia. Further, the impact on autonomic measures was explored and relations between the nocebo response and personality traits were investigated. Nocebo-hyperalgesia as indicated by the subjective measure was successfully induced in part of the sample, independent from contingency awareness. Successfully conditioned subjects compared to non-successfully conditioned subjects showed to be habitually less anxious, received higher stimulus intensities despite comparable subjective sensation, and demonstrated increased heart rate and decreased HRV parameters. Motivational style and suggestibility were not related to the nocebo response.

Study three investigated neural correlates of uncontrollability-induced pain increase. During controllable pain trials, subjects showed temporal summation, but adapted during controllable warm trials, as indicated by the behavioral measure. During the uncontrollable pain condition, subjective intensity ratings increased over the course of the individual trials, despite subjects received the

identical nociceptive input that they had regulated to feel constant in the controllable condition. The additional pain increase in the pain trials, induced by uncontrollability, was mirrored in increased activation of pain processing brain regions, such as thalamus, insula, SII, and ACC. Importantly, activity in perigenual ACC and PAG drove the uncontrollability-induced pain increase. These results suggest that the loss of control leads to activation of a pro-nociceptive circuitry also assumed to play a role in placebo and nocebo effects that involve the pain modulatory regions PAG and pACC.

In summary, these studies demonstrated a) the powerful impact of psychological factors, such as learning and uncontrollability, on pain perception, and b) proved the benefit of a multidimensional assessment of pain perception and its correlates. These results improve our understanding of pain facilitatory processes and have important implications for therapeutical interventions in pain conditions. They can further promote research in other fields, for example concerning the role of classical conditioning and neural processes in chronic pain.

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Appendix

Supplementary Figure I:

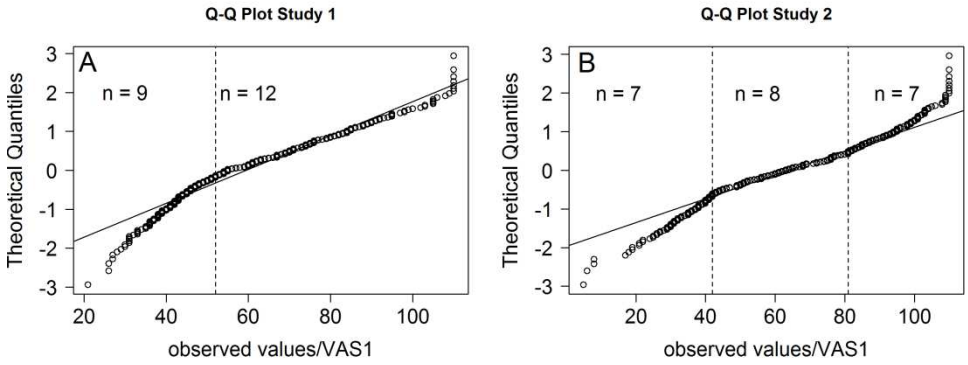

Figure SI: Displayed are quantile-quantile (QQ) plots of the subjective ratings (VAS_1) of painful trials during the learning phases of Study 1 (A) and Study 2 (B). In order to classify subjects into different groups we put 2 (Study 1) or 3 (Study 3) straight lines through the distribution of single trials and allocated the subjects (n) into one group (of two or accordingly three groups) according to their mode within trials, as indicated by the dashed lines.

Supplementary Figure II:

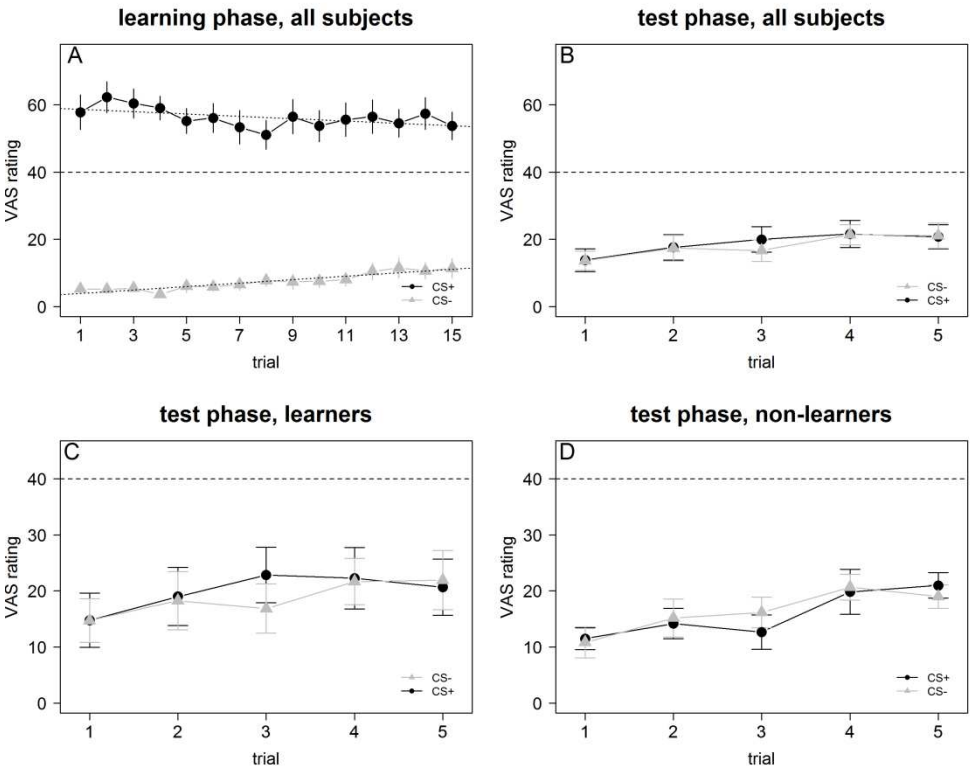

Figure SII: Subjective rating (VAS_2 after the behavioral task) throughout Study 1. Depicted are subjective ratings (mean and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and the test phase (B) of the whole sample (N = 21) and during the test phase of the learner subgroup (C; N = 13), and non-learner subgroup (D; N = 8). The dashed line depicts the pain threshold (VAS = 40). Linear mixed model analyses revealed that both CS were rated differently during the learning phase (main effect 'CS': $F(1, 52.01) = 476.7$, $p < .001$) and remained stable over time (main effect 'time': $F(14, 181.9) = 0.39$, $p = .976$; interaction effect 'CS' x 'time': $F(14, 273.1) = 1.1$, $p = .357$). During the test phase, the rating of trials cued with both CS was not different (main effect 'CS': $F(1, 44) = 0.87$, $p = .356$), but increased over time (main effect 'time': $F(4, 78.6) = 3.93$, $p = .006$), which was the same for both CS (interaction effect 'CS' x 'time': $F(4, 81.4) = 0.93$, $p = .452$). When restricting the analyses to the learner subgroup, subjects did not differentiate between trials cued with either CS (main effect 'CS': $F(1, 33.2) = 1.97$, $p = .169$) and the ratings did no change over time (main effect 'time': $F(4, 56.8) = 1.87$, $p = .128$; interaction effect 'CS' x 'time': $F(4, 58.3) = 2.24$, $p = .076$).

Supplementary Figure III:

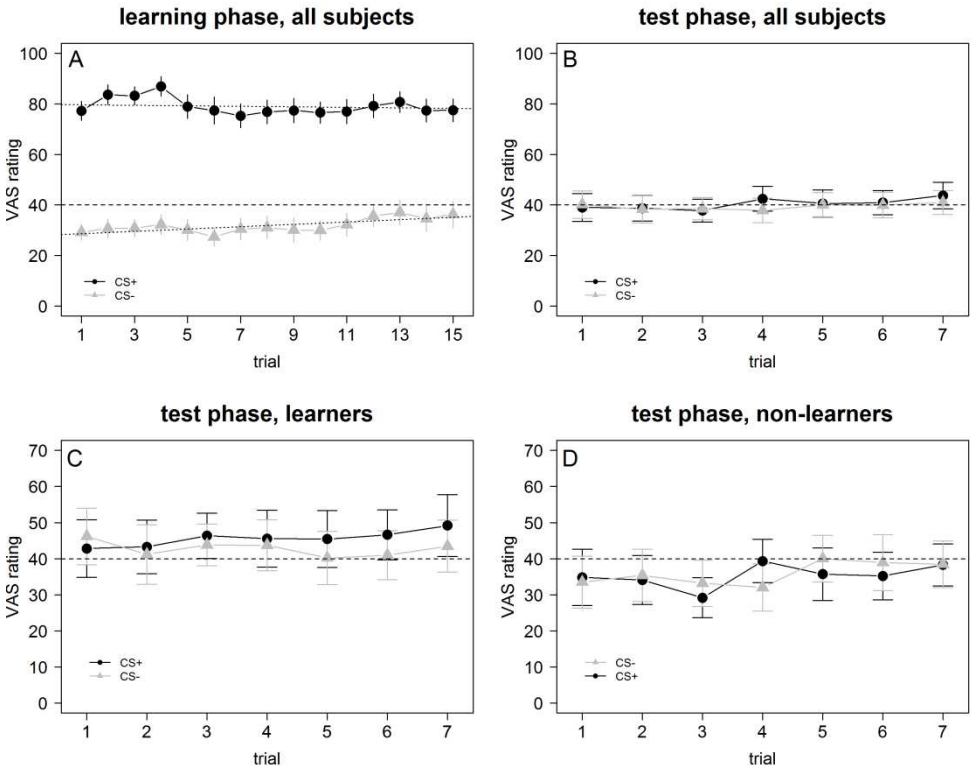

Figure SIII: Subjective ratings (VAS_2 after the behavioral task) throughout Study 2. Depicted are subjective ratings (means and standard errors of mean) of trials cued with CS+ (black) and CS– (grey) during the learning phase (A) and test phase (B) of the whole sample (N = 22) and during the test phase of the learner subgroup (C; N = 11) and non-learner subgroup (D; N = 11). The dashed line depicts the pain threshold (VAS = 40). Analyses showed that during the learning phase, the subjects perceived moderately painful trials as more painful than mildly painful trials (main effect 'CS': $F(1, 523.3) = 1784.4$, $p < .001$), which did not change over time (main effect 'time': $F(14, 567.8) = 0.72$, $p = .753$; interaction effect 'CS' x 'time': $F(14, 523.3) = 0.8$, $p = .674$). During the test phase, the subjects did not rate trials differently when cued with either CS (main effect 'CS': $F(1, 189.5) = 0.91$, $p = .341$) and there was no change over time (main effect 'time': $F(6, 201.8) = 0.37$, $p = .0.9$; interaction effect 'CS' x 'time': $F(6, 189.4) = 1.02$, $p = .417$). The learner subgroup rated trials cued with CS+ as more painful than with CS– (main effect 'CS': $F(1, 94.8) = 4.8$, $p = .031$), which did not change over time (main effect 'time': $F(6, 100.5) = 0.16$, $p = .986$; interaction effect 'CS' x 'time': $F(6, 94.8) = 0.9$, $p = .502$).

Supplementary Figure IV:

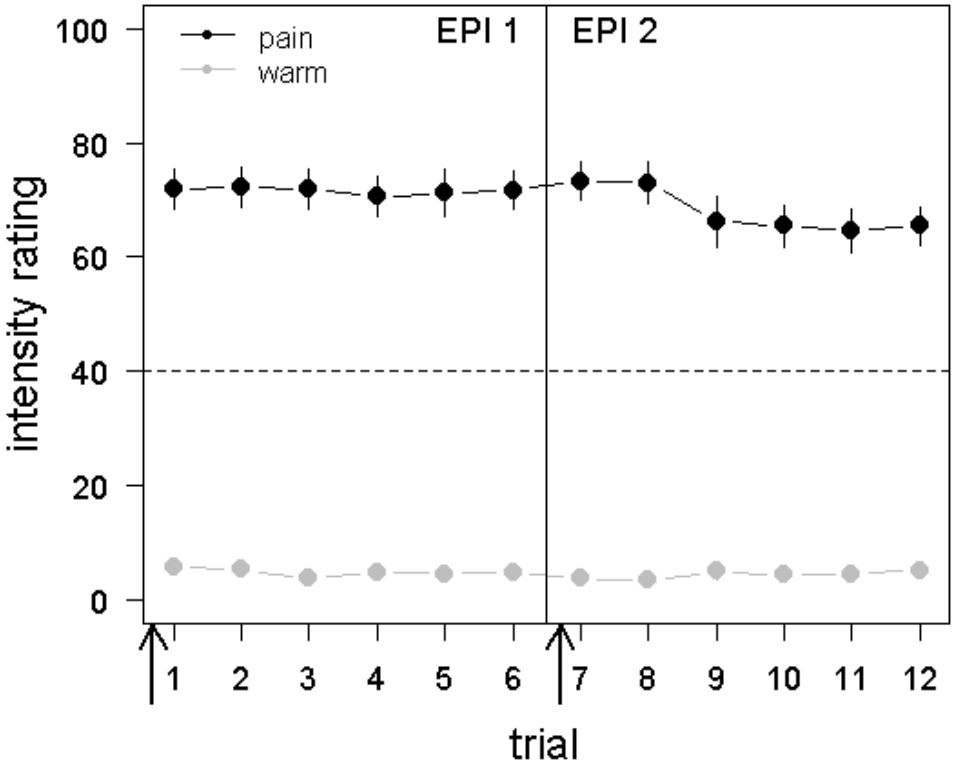

Figure SIV: Intensity ratings of the uncontrollable trials, averaged across participants with standard error of means. The arrows pointing at the x-axis indicate the time points of the controllable trials (6 trials each). The ratings remained stable across the uncontrollable trials, which is supported by the results of the mixed model analysis (interaction effect Trial x Intensity: $F(5, 514) = 0.7$, $p = 0.626$). The dashed line depicts the pain threshold (VAS = 40).

Supplementary Table I: Brain responses to painful vs. warm stimulation, irrespective of the task (controllable and uncontrollable painful > controllable and uncontrollable warm).

brain region	cluster size (# of voxels)	Z score peak	later-ality	MNI peak coordinates in mm		
				x	y	z
cluster spanning the following regions:	32627	6.73	bl	56	0	0
insula		6.26	bl	34	2	8
SII		6.24	bl	46	-2	2
ACC		4.71	bl	4	10	44
thalamus		6.21	bl	18	-16	12
cerebellum		6.06	bl	-30	-60	-34
superior temporal gyrus		6.73	bl	56	0	0

One big cluster spanning several typical pain processing areas was significantly more activated by painful compared to non-painful stimulation (significant on a whole brain-level, voxel-based threshold Z = 2.3 and cluster-based threshold $p < 0.05$). Local maxima within the cluster are given for individual anatomical areas. SII, secondary somatosensory cortex; ACC, anterior cingulate cortex; bl, bilateral.

Supplementary Table II: Brain responses to uncontrollable painful stimulation compared to controllable painful stimulation (uncontrollable painful > uncontrollable painful).

brain region	cluster size (# of voxels)	Z score peak	later-ality	MNI peak coordinates in mm		
				x	y	z
cluster spanning the following regions:	2163	4.99	bl	18	-16	10
PAG		3.99	r	16	-26	-16
thalamus		4.99	bl	18	-16	10
premotor cortex	1059	5.27	r	22	4	56
precuneus cortex	407	4.17	bl	-16	-66	26
cluster spanning the following regions:	11345	6.25	bl	-10	-100	-6
visual cortex		6.25	bl	-10	-100	-6
cerebellum		5.95	bl	-12	-56	-50

Listed are brain areas in which activation was significant on a whole brain-level, voxel-based threshold Z = 2.3 and cluster-based threshold $p < 0.05$. Please note that local maxima are given as peaks if a significant cluster encompassed more than one region. PAG, periaqueductal gray; bl, bilateral; r, right.

Supplementary Table III: Brain activation correlated with sensitization in the controllable condition.

brain region	cluster size (# of voxels)	Z score peak	later-ality	MNI peak coordinates in mm		
				x	y	z
cluster spanning the following regions:	1463	3.98	bl	-6	-30	38
posterior cingulate cortex		3.98	bl	-6	-30	38
precuneus cortex		3.93	bl	-2	-44	54
cluster spanning the following regions:	*682*	3.35	bl	-14	-38	-8
parahippocampal gyrus		3.35	l	-14	-38	-8
cuneal cortex		3.17	r	8	-84	42
visual cortex		3.15	l	-10	-66	12
cluster spanning the following regions:	580	3.56	r	30	-16	58
premotor cortex		3.56	r	30	-16	58
SI		3.36	r	48	-20	50
MI		3.26	r	46	-10	34
cluster spanning the following regions:	319	3.34	r	18	-38	-4
parahippocampal gyrus		3.34	r	18	-38	-4
lingual gyrus		3.09	r	16	-38	-12
visual cortex	2156	3.86	bl	18	-100	-2

Listed are brain areas in which activation was significant on a whole brain-level, voxel-based threshold Z = 2.3 and cluster-based threshold $p < 0.05$. Please note that local maxima are given as peaks if a significant cluster encompassed more than one region. SI, primary somatosensory cortex; MI, primary motor cortex; bl, bilateral; r, right; l, left.

Supplementary Table IV: Brain activation reflecting the additional sensitization in the uncontrollable condition.

brain region	cluster size (# of voxels)	Z score peak	later-ality	MNI peak coordinates in mm		
				x	y	z
cluster spanning the following regions:	1275	3.67	bl	6	24	52
anterior cingulate cortex		3.29	bl	4	34	18
premotor cortex		3.67	r	6	24	52
cluster spanning the following regions:	1084	3.75	r	44	18	-4
insula		3.52	r	44	8	-8
frontal operculum		3.75	r	44	18	-4
temporal pole		3.7	r	50	18	-8
cluster spanning the following regions:	689	3.92	l	-44	14	-4
insula		3.93	l	44	14	-4
SII		2.9	l	-48	-8	6
temporal pole		2.89	l	-54	10	-6
cluster spanning the following regions:		3.31	bl	16	-8	8
thalamus		3.31	bl	16	-8	8
pallidum		3.11	r	12	-8	-6
cluster spanning the following regions:	1440	5.04	bl	14	-84	-16
occipital fusiform gyrus		5.04	r	14	-84	-16
visual cortex		3.63	bl	6	-92	-6

Listed are brain areas in which activation was significant on a whole brain-level, voxel-based threshold Z = 2.3 and cluster-based threshold $p < 0.05$. Please note that local maxima are given as peaks if a significant cluster encompassed more than one region. SII, secondary somatosensory cortex; bl, bilateral; r, right; l, left.

Eidesstattliche Versicherung

Eidesstattliche Versicherung gemäß § 9 Absatz 1 Buchstabe e) der Promotionsordnung der Universität Mannheim zur Erlangung des Doktorgrades der Sozialwissenschaften:

1. Bei der eingereichten Dissertation mit dem Titel „Cognitive and Behavioral Context of Pain Facilitation - Nocebo Conditioning and Uncontrollability-Induced Sensitization handelt es sich um mein eigenständig erstelltes Werk.
2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtliche Zitate aus anderen Werken als solche kenntlich gemacht.
3. Die Arbeit oder Teile davon habe ich bisher nicht an einer Hochschule des In- oder Auslandes als Bestandteil einer Prüfungs- oder Qualifikationsleistung vorgelegt.
4. Die Richtigkeit der vorstehenden Erklärung bestätige ich.
5. Die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt.

Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Mannheim, 04.02.2014 Anne-Kathrin Bräscher