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The role of learning mechanisms on pain perception and chronification.

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1. Introduction

The relevance of pain and pain-related learning for the (human) organism already concerned ancient scholars. Thus, for example, Epicurus and Marcus Tullius Cicero wrote:

"The appearance of some physical pain uses caution in similar cases."

Epicurus of Samos (341 – 270 BC, Greek philosopher)

"Those who have suffered pain remember it."

Marcus Tullius Cicero (106 – 43 BC, Roman orator and politician)

These observations become particularly relevant when it comes to chronic pain disorders. Patients suffering from chronic pain typically exhibit strong avoidance behaviors towards certain movements or situations that they once have experienced in a painful context and that they therefore often believe, will elicit or exacerbate their pain. Thus, learning behaviors and memory processes have a long history of being discussed in the context of chronic pain (Flor and Turk, 2011; Linton et al., 1984). On behalf of these discussions, the study that this doctoral thesis is based on, compared pain-related emotional learning processes and their modulation by pharmacologically induced stress in patients suffering from non-specific chronic back pain and age- and gender-matched, pain-free healthy volunteers. In the following introductory sections, the term *pain* will first be defined, before the differences between acute and chronic pain will be elaborated. Further, learning mechanisms in the context of pain and the role of classical conditioning, specifically fear conditioning, in chronic pain disorders will be emphasized. Finally, the reciprocal relationship of pain and stress and its influence on pain-related (fear) learning will be described.

1.1. Pain

When it comes to describing pain, we soon realize that this is not as simple as it possibly seems. Pain is a complex and subjective sensory, but also emotional phenomenon involving sensory (*nociceptive*) input that is initially registered via so-called *pain receptors* (i.e., *nociceptors*) and further transmitted through *pain fibers* to the *central nervous system (CNS)*. Simultaneously, nociceptive input is modulated by psychological factors including attention, mood or our condition of the day. This complexity leads to inter- and intra-individual differences in terms of how pain is perceived, processed, dealt with, and finally, described. Therefore, the *International Association for the Study of Pain (IASP)* published the following definition of pain:

"An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." (Raja et al., 2020).

With this definition, the IASP does not reduce pain to obviously identifiable tissue damage and thus, definable causes, but also considers it to be existent even in the absence of physiological causes. The terms *pain* and *nociception* must therefore be distinguished from each other, as pain cannot always be explained solely by sensory neuron activity, but can be triggered by emotional mechanisms such as (pain-related) fear or expectations as well. Pain should thus be taken seriously when the patient reports it.

1.1.1. Acute pain

Despite its unpleasantness, acute pain is a highly relevant and evolutionarily hardwired, biological warning signal that alerts us to existing or potential harmful situations, and thus contributes to the maintenance of the organism's integrity.

As described in Bell (2018), an acute painful somatic sensation is initiated by the excitation of free nerve endings of sensory neurons of the skin, the so-called *pain receptors* or *nociceptors*, which transmit the *nociceptive input* from the *peripheral nervous system (PNS)*

to the CNS. In response to a noxious stimulus (i.e., thermal, chemical, or mechanical), nociceptors can elicit an action potential that is transmitted through the *sensory neuron* to the *posterior dorsal horn* of the spinal cord. These neurons are called *pain fibers* and are divided into myelinated, fast-conducting *Aδ fibers*, transmitting the so-called *first pain* (not too painful, but essential for the organism to quickly locate the painful sensation and initiate protective behavior) and non-myelinated, slow-conducting *C fibers*, transmitting the *second pain* (more severe and highly intensive, but more difficult to localize). Once the action potential reaches the spinal cord, it is transferred from the PNS to the CNS and subsequently transmitted via various ascending pathways to the *brainstem*, *midbrain* or *thalamus*, from where it is projected and distributed to a widespread cortical network of somatosensory, insular, cingulate, frontal and parietal areas (Bell, 2018; Tracey and Mantyh, 2007).

Once a nociceptive signal from this *ascending pain pathway* enters the somatosensory cortex, the *descending pain modulatory system* is activated. Especially the *periaqueductal grey*, but also the *rostroventromedial medulla* play a key role in the modulation of nociception, particularly in mediating endogenous analgesia by reducing pain signaling through neuronal inhibition (i.e., top-down modulation of pain) in order to allow an appropriate response to the source of pain (Ossipov et al., 2014).

1.1.2. Chronic pain

Throughout the healing process, acute pain typically diminishes. Sometimes, however, pain persists despite the fact that a physiological cause can no longer be identified. In this case, its warning function got lost and it developed to a “disease in its own right”, referred to as chronic pain (Clauw et al., 2019), which is defined as “persistent or recurrent pain lasting longer than three months” (Treede et al., 2015).

The development of persistent pain may be mediated by peripheral changes following tissue or nerve damage, but also by changes in the transmission and processing of nociceptive stimuli, leading to painful perception of non-noxious stimuli (i.e., *allodynia*), or increased pain sensitivity / decreased pain thresholds (*hypersensitivity*) (Baller and Ross, 2017;

Dickenson, 2008). Currently, alterations in learning and memory processes are discussed and changes in emotional and motivational brain circuits were found to be related to chronic pain (Apkarian et al., 2011; Baliki et al., 2012). However, whether these are the cause or the consequence of chronic pain, is still not known.

It is therefore not surprising that chronic pain is a highly complex disease typically involving multiple and often interrelated causes (e.g., mechanical, physiological, and psychological), which makes its treatment a major challenge for practitioners. Patients commonly report a long history (often years) of seeking help, which obviously creates a great psychological and economic burden. Cohen et al. (2021) summarized that the worldwide prevalence of chronic pain varies between 11 and 40% with increased numbers in low-income countries.

Chronic back pain. Among chronic pain, chronic back pain (CBP) is one of the most frequently reported disorders (Komarahadi et al., 2006) and one of the leading diseases contributing to years lived with disability worldwide (Murray et al., 2013; von der Lippe et al., 2021). Thus, it is further one of the top reasons for work incapacity or early retirement, resulting in a tremendous economic burden not only for the patients, but also for their families and the state (Kent and Keating, 2005; RKI, 2014). With a prevalence of around 20% (von der Lippe et al., 2021), CBP is among the most frequent health problems of the German population that often comes along with reduced daily activity, diminished health-related quality of life (Lidgren, 2003), and mental comorbidities (i.e., depression, anxiety, and insomnia) (Singhal et al., 2021). Due to its typical non-specific nature (i.e., no specific nociceptive cause is identifiable), a common problem of its treatment are rash decisions for pharmacological treatments (e.g., excessive opioid prescriptions) and/or recommendations for surgical interventions, which often target only the symptomatology rather than the underlying cause (Maher et al., 2017). Thus, the need for improved treatment strategies becomes increasingly urgent. However, this is only possible through a better understanding of the underlying mechanisms promoting the development and maintenance of CBP.

1.2. Learning mechanisms in the context of pain

One important factor that has recently been discussed to promote the development and maintenance of chronic pain is *pain-related learning*. For the health and survival of an organism, it is essential to properly distinguish between cues that (potentially) pose a threat to the organism's integrity (i.e., *threat cues*) and those that signal safety (i.e., *safety cues*), and, furthermore, to develop adaptive responses based on these experiences (Vlaeyen, 2015). For instance, after a painful experience during a movement (e.g., due to an injury), it may be beneficial to develop protective behavior through pain-related learning and temporarily avoid that movement to promote the healing process. However, it is equally important to "unlearn" these associations through *extinction learning* when they are no longer required (e.g., when the physical trauma has recovered) in order to deactivate the protective mechanisms and return to normal behavior. In chronic pain, not extinguished "pain memory" rather than actual nociceptive input is discussed to cause the experienced pain (Flor and Turk, 2011). As stated in the fear avoidance model of chronic pain of Vlaeyen and Linton (2000), patients often fall into a vicious circle of anxiety and avoidance: Starting with *pain catastrophizing*, patients often develop *pain-related fear*, which can lead to avoidance behavior and hypervigilance towards physical sensations. At the end, this often results in disability, disuse, and depression for the patients, which further maintains the pain experience and reinforces this vicious circle. Non-catastrophizing patients commonly do not develop strong pain-related fear. Thus, repeated painless experiences usually lead to fast recovery (Vlaeyen and Linton, 2000).

1.3. Classical conditioning as a tool in pain research

As described in the following chain of thoughts adapted from Flor (2012), a recurrent experience of pain could be the crucial process in the transition from acute to chronic pain through adaptive psychological/behavioral and physiological pain-related learning processes similar to *classical conditioning*. According to Pavlov (1927), in classical conditioning a neutral, biologically irrelevant stimulus is recurrently paired with a biologically relevant stimulus (i.e., *unconditioned stimulus, US*) that typically elicits an *unconditioned response (UR)*. Thus, the former neutral stimulus turns into a *conditioned stimulus (CS)*, and the UR

is also elicited by the CS alone, which turns the UR into a *conditioned response (CR)*. These processes are particularly relevant in chronic pain research as, e.g., patients with non-specific CBP often associate particular movements, e.g., bending, with their pain (US) that were performed when the pain initially emerged. In turn, the former neutral movement may become a CS for the patients that they believe to predict/trigger their pain. Later, the mere thought of this movement can induce pain-related fear and muscle tension (both CRs) and further lead to avoidance behavior and other pathologies that were described above.

For the investigation of pain-related learning processes, classical conditioning paradigms can be used according to the overview article of Lonsdorf et al. (2017): this entails an initial *acquisition training* phase where repeated paired CS-US presentations are used to provoke CRs on the CS. Thus, a (pain-related) *acquisition memory* is developed through the process of associative learning about the US-predictive character of the CS. Classically, neutral visual stimuli such as geometric figures, different colored lights, or human faces are used as CS (Merz et al., 2020, 2016; Schmidt et al., 2020), but auditory, tactile, olfactory, or gustatory stimuli can also be considered. In pain-related conditioning, CS are commonly paired (together, preceding or overlapping) with aversive somatic (i.e., heat), visceral (i.e., rectal distension), or an electrical stimulus as painful US (Koenen et al., 2018; Schmidt et al., 2020).

The acquisition training is usually followed by an *extinction training* phase, including only CS but no US presentations. Thus, CRs typically gradually attenuate and new associations with the CS develop (i.e., *extinction memory*) through *extinction learning*, which further competes and usually inhibits the retrieval of the original acquisition memory (Hartley and Phelps, 2012). This is, as described earlier, highly relevant for a healthy and sustainable recovery process and thus, to prevent the development of, e.g. chronic pain. However, chronic pain, if already present, is also widely treated through exposure therapies, that are primarily based on extinction learning mechanisms (Hartley and Phelps, 2012).

In both experimental and clinical settings, it is repeatedly noticed that extinction memory is not always retrieved during later exposure to the CS and sometimes *spontaneous recovery* of the acquisition memory occurs (e.g., relapse of the chronic pain). This relapse phenomenon

can be investigated using a *recall test* phase (usually at least 24 h after extinction training), where, again, only CS but no US are presented.

Observations from clinical settings further indicate that an unexpected exposure to the original US, although in another situation (e.g., a painful sensation in the same area without performing the same movement), can reinstate the acquisition memory and cause a relapse. Therefore, in a *reinstatement manipulation* phase, the original US is presented without any predicting CS, followed by a *reinstatement test* phase, where only CS are presented.

Lonsdorf et al. (2017) further state that, as a form of classical and a part of pain-related conditioning, fear conditioning can be used as a valuable approach to investigate (pain-related) learning processes in different organisms and populations, and further to translate results from different animal models to humans, clinical populations, and back. They propose several outcome measures to capture learning processes during fear conditioning: (i) physiological measures such as skin conductance responses (SCRs) to assess the emotional arousal to different stimuli, (ii) behavioral measures like self-reported valence ratings to capture the un-/pleasantness of the CS, or (iii) US-expectancy and contingency ratings to verify the awareness of the CS-US coupling. Since the emotional component seems to play a crucial role in chronic pain disorders, the focus of this project was primarily on emotionally relevant measures, i.e., valence ratings and SCRs. Therefore, in the following, this thesis refers to emotional learning and conditioning as a modality of fear learning.

As described in Meulders (2020), the processes of fear and emotional learning have been well studied in healthy volunteers over the past 100 years. Not only the terminology was standardized (Lonsdorf et al., 2017), but also different paradigms and US were tested, established, and optimized to study not only the acquisition and extinction of CRs to a single cue, but also differential (pain-related) learning of different cues either predicting the occurrence of a US as a threat (CS+) or its absence as a safety signal (CS-). Further, different US or pain modalities, e.g., somatic vs. visceral pain (Koenen et al., 2018) or the application of the same US to different body parts, e.g. hand vs. face (Schmidt et al., 2020) have been investigated and found to differentially affect pain-related differential (i.e. threat vs. safety) learning. Further, greater conditioning effects were found for aversive compared to appetitive

learning (van der Schaaf et al., 2022). Beyond that, models were developed and established to study relapse (Haaker et al., 2014) or generalization (Dunsmoor and Murphy, 2015) phenomena, as these are thought to be critical for the maintenance of chronic disorders (e.g., anxiety, pain, etc.). However, these processes and underlying mechanisms also need to be studied in clinical populations (e.g., patients with chronic pain) in order to use the knowledge of potential alterations for improving medical care. Although research interest has increased in this field, studies are still scarce and findings are mixed (Harvie et al., 2017). Mainly, impaired threat and/or safety learning was reported, e.g., in patients suffering from fibromyalgia (Meulders et al., 2017, 2015), chronic neck (Harvie et al., 2020) or hand pain (Meulders et al., 2014). Further, patients with chronic unilateral hand pain revealed reduced contingency awareness. However, Icenhour et al. (2015) observed stronger pain-related learning of safety cues in patients with irritable bowel syndrome, and elevated conditioned muscular responses in patients with CBP were reported by Schneider et al. (2004) and Klinger et al. (2010), while findings on extinction learning point into opposite directions. In general, findings on extinction-related phenomena in chronic pain populations are still missing and sample sizes are rather small ($N \leq 30$) considering the typically heterogeneous character of chronic pain or clinical populations as already pointed out in Schlitt et al. (2021).

1.4. The role of stress in fear conditioning and chronic pain

A prominent key player in fear conditioning, but also acute and chronic pain is *stress*. The *World Health Organization* describes it as "any type of change [or situation] that causes physical, emotional or psychological strain [resulting in] bodily [up to behavioral] responses to anything that requires attention or action." (WHO, 2021).

1.4.1. The relationship between stress, chronic pain, and learning behavior

Timmers et al. (2019) outline the importance of the close and reciprocal relationship between stress and (chronic) pain in their review as follows: an acute painful situation typically triggers *the hypothalamic-pituitary-adrenal (HPA) axis*, which is also referred to as the *stress*

response system (Ulrich-Lai and Herman, 2009). Thus, in chronic pain, the HPA axis might be permanently triggered, which possibly leads to its dysregulation (Woda et al., 2016). Furthermore, however, acute stress is known to affect pain perception, e.g., of experimentally applied painful stimuli (Timmers et al., 2018) and to increase pain intensity in patients with chronic pain (Fischer et al., 2016). Hence, stress is discussed to contribute to the development and maintenance of chronic pain. Timmers et al. (2019) further proposed an adapted version of the fear avoidance model where four possible intersections of stress, fear learning and chronic pain are highlighted: first, they discuss a positive influence of stress on threat processing and perception by increasing attention to a (painful) stimulus or (pain-related) situation that signals actual or potential tissue damage. Further, they argue that the activation threshold of the HPA axis, and thus, the release of a stress response could be downregulated by pain-related cognitions (e.g., *pain catastrophizing* and *fear of pain*). Beyond that, stress is considered to be a primer to inflexible and habitual immediate pain control rather than to flexible value-based behavior. Finally, they discuss the influence of stress on threat learning by stress-induced increases in threat perception and pain control behavior.

1.4.2. Stress in human extinction learning studies and the role of its timing

As described above, extinction learning processes are an essential component of exposure therapies, which are also applied in chronic pain treatment. Therefore, research on the influence of stress on extinction learning and the robustness of extinction memory is highly relevant. First studies on healthy human volunteers already demonstrated a differential modulating influence of stress on extinction learning dependent on the timing of its application (Hamacher-Dang et al., 2015; Merz et al., 2020; Raio et al., 2014). With the *STaR (Stress Timing affects Relapse) model*, Meir Drexler et al. (2019) introduced a theoretical model on the modulatory and time-dependent role of stress on extinction learning and relapse phenomena. They first postulate that stress induction before extinction fosters strong context-independent, more generalized extinction memory and thus, reduces the risk of relapse. Further, stress application after extinction training is discussed to promote strong context-dependent extinction memory, which means that the risk of relapse would also be context-

dependent. Finally, they propose stress induced prior to extinction retrieval impedes extinction memory retrieval, which leads to an increased risk of relapse.

Primarily, three approaches are used to examine the modulatory influence of stress on extinction learning and relapse phenomena by triggering the HPA axis: (1) During the *Socially Evaluated Cold Pressure Test (SECPT)*, participants are told that they are videotaped and their facial expressions are analyzed while they need to hold their hand in ice water for as long as possible (Hamacher-Dang et al., 2015; Schwabe et al., 2008). (2) The *Trier Social Stress Test (TSST)* comprises a short oration and a mental arithmetic task that must be solved in front of a jury (Kirschbaum et al., 1993). (3) Further, *hydrocortisone* as a *glucocorticoid (GC)* and an end product of the HPA axis is used to pharmacologically induce stress in the context of (pain-related) fear conditioning studies (Hagedorn et al., 2021; Merz et al., 2013). In the present study, the hydrocortisone administration approach was chosen since, compared to the SECPT or the TSST, salivary cortisol levels are typically higher, thus suggesting an increased stress response. Furthermore, the administration of hydrocortisone as a tablet is feasible and does not need complex preparations or setups as compared to the other approaches. However, at the end it might be a matter of taste which approach is chosen.

1.5. Research questions, hypotheses, and aims of this study

Firstly, this study investigated potential alterations in pain-related learning processes (i.e., acquisition and extinction learning) in n=62 patients suffering from non-specific CBP and n=61 age- and gender-matched pain-free healthy participants (healthy controls, HC). Further, the modulatory role of pharmacologically induced stress on the retrieval of extinction memory and the relapse of former acquired pain-related fear was examined in both groups by administering 20mg of hydrocortisone or an inert substance (i.e., placebo) in a double-blind, randomized, controlled design before an extinction retrieval test. Participants performed a 2-day classical differential conditioning paradigm with geometric figures as visual cues (CS) that predicted either the occurrence (CS+) or omission (CS-) of a phasic heat pain stimulus of moderate to high pain intensity (US). Individual CS valence ratings were assessed as a self-reported behavioral measure for implicit emotional learning, CS-US contingency ratings for explicit learning, and SCRs as a physiological measure. Beyond that pain intensity ratings were assessed in order to check for equivalent pain perception in both groups and exploratory analyses were performed to investigate effects of psychological, pain- and cortisol-level-related parameters on emotional and extinction learning. A priori, the following hypotheses were made:

1. Patients with CBP will reveal impaired pain-related threat and safety learning (i.e., impaired differential learning) as compared to HCs.
2. The administration of hydrocortisone before extinction retrieval will impair extinction memory retrieval and lead to stronger relapse of pain-related associations in HCs.
3. Patients with CBP will generally show impaired retrieval of extinction memory and stronger relapse of former acquired pain-related associations.
4. These effects will be enhanced in patients with CBP that received hydrocortisone.

2. Methods

In this section, methods will be described that were used to investigate (i) pain-related learning processes in patients suffering from non-specific chronic back pain (CBP) and age- and gender matched, pain-free healthy participants (HCs) as well as (ii) the influence of stress on the retrieval of acquisition and extinction memory in both experimental groups based on Schlitt et al. (2021) and the previously mentioned manuscript by Schmidt & Schlitt et al. that is currently under peer-review at *The Journal of Pain* (see *Publications*).

After a brief description of the two experimental groups, the experimental design and procedures are presented. Thereafter, the classical differential conditioning paradigm including the stimuli and experimental phases is introduced along with the outcome measures that were analyzed within this study. Finally, the analysis strategy is described at the end of section.

2.1. Experimental groups and their characteristics

2.1.1. Recruitment

Based on previous studies examining pain-related learning in patients with chronic pain (Meulders et al., 2017, 2014), a required sample size of n = 63 for each group was calculated to discover differences in differential acquisition and extinction learning between groups. Therefore, the R package '*pwr'* (Champely et al., 2020) was used including the subsequent parameters: d = 0.5, α = 0.05, 1-β = 0.80. Main sources for the recruitment of N = 141 volunteers where the working group's internal data base of former research participants, newspaper advertisements, postings at the campuses of the University Medicine Essen and the University Duisburg-Essen, and the local back pain center at the University Medicine Essen (head: Prof. Dr. med. Ulrike Bingel). Before inclusion, all participants underwent a telephone screening performed by trained study personnel in order to screen for eligibility. General inclusion criteria for both groups comprised age >18 and <80 years, no acute infection or participation in trials with investigational medicinal products during the past 12

weeks, normal or corrected-to-normal vision, and no alcohol intake in the last 24 hours (according to self-report). Specific inclusion criteria for both experimental groups are listed below. Written and verbal informed consent for study participation and for publication of anonymized data was obtained from all participants. They were free to withdraw at any time and received a reasonable remuneration for participation. The local Ethics Committee of the Medical Faculty of the University of Duisburg-Essen (University of Duisburg-Essen, Germany; 16-7248-BO) approved this study and had no objections to its conduct.

2.1.2. Patients with non-specific chronic back pain

The included patient population comprised n = 67 patients suffering from non-specific CBP, defined as remitting or persistent pain >12 weeks according to the European guidelines on CBP (Airaksinen et al., 2006). Thus, patients with specific spinal pathologies, postsurgical, nerve root, or post-traumatic pain were excluded. Additional patient-specific exclusion criteria covered malignant (in the last 5 years), or mental diseases (e.g., schizophrenia, major depression, psychosis), and opioid intake >100 mg morphine equivalent per day. Further, it was mandatory that any treatment (pharmacological, psychological, or physiotherapeutical) remained unchanged for at least 3 weeks before study participation in order to control for the influence of treatment effects on the study outcomes. For eligible patients, an additional on-site screening by physicians specialized in pain medicine (Prof. Dr. med. U. Bingel and Dr. med. J. Kleine-Borgmann) was implemented.

2.1.3. Pain-free healthy participants

The control group included n = 74 pain-free healthy participants that did not report any actual or history of pain-related, internal, neurological, psychological, dermatological, or malignant diseases (e.g., cancer), or the usage of recreational drugs. Individuals that revealed elevated levels of depression or anxiety according to the DASS (Depression Anxiety Stress Scales) questionnaire (Nilges and Essau, 2015) were later excluded from data analysis (cut-off values: depression = 10, anxiety = 6, stress = 10;). To whom applicable, medication had to

remain stable during the past 3 months before participation (e.g., hormonal contraceptives, allergy medication, etc.) and the intake of analgesics <24 hours before study participation led to exclusion within the control group.

2.1.4. Final sample for data analysis

After data acquisition, n=5 patients with CBP and n = 13 HCs were excluded from the final data analysis due to the following reasons: (i) Pain intensity ratings of n = 3 patients with CBP and n = 2 HCs did not match the envisaged level during calibration. (ii) Mean pain intensity ratings of n = 2 patients with CBP and n = 1 HC were below 30 on the 0-100 Visual Analogue Scale (VAS; used for the rating procedure) during the experimental task. (iii) After evaluation of the DASS questionnaire, clinically relevant levels of anxiety or depression were found in n=2 HCs, and (iv) n = 8 HCs lost eligibility after inclusion. Thus, the final sample for the analysis of study day 1 comprised n = 62 patients with CBP (n = 18 male, age: 34.56 ± 13.37 (M ± SD) years) and n = 61 HCs (21 male, age: 33.80 ± 11.83 years).

Additionally, n = 2 patients with CBP and n = 2 HCs were ruled out from the analysis of study day 2: (i) Mean pain intensity ratings of n = 1 HC remained below the envisaged level of 30 on the 0-100 VAS within the reinstatement manipulation phase. (ii) Technical issues with the thermal heat pain stimulator occurred in n=2 patients with CBP and n = 1 HC. The final sample for the analysis of study day 2 thus comprised n = 60 patients with CBP (17 male, age: 34.40 ± 13.40 years) and n = 59 HCs (21 male, age: 33.80 ± 11.80 years).

Demographic information and pain-related patient characteristics for both analyzed samples are listed in *Table 1 – Characteristics of patients with CBP and HCs for the analyzed samples of study day 1 and 2, respectively*.

Table 1. Characteristics of patients with CBP and HCs for the analyzed samples of study day 1 and 2, respectively.

	Study Day 1		**Study Day 2**	
	CBP *(N = 62)*	**HC** *(N = 61)*	**CBP** *(N = 60)*	**HC** *(N = 59)*
Demographic properties				
Age (years), *M ± SD [Range]*	34.6 ± 13.4 [16–69]	33.8 ± 11.8 [19–70]	34.4 ± 13.4 [19–69]	33.8 ± 11.8 [19–70]
Female/male, *n (%)*	44/18 (71.0/29.0)	40/21 (65.6/34.4)	43/17 (72.7/27.3)	38/21 (64.4/35.6)
Educational background, *n (%)*				
Basic (≤10 years)	12 (19.4)	7 (11.5)	11 (18.3)	6 (10.2)
High school (13 years)	29 (46.7)	40 (65.5)	28 (46.7)	39 (66.1)
University (>13 years)	21 (33.9)	14 (23.0)	21 (35.0)	14 (23.7)
Treatment (only Study Day 2)				
Placebo/Hydrocortisone *n (%)*	-	-	30/30 (50.0/50.0)	26/33 (44.1/55.9)
Pain-related traits, *M ± SD [Range]*				
Pain duration (years)	9.79 ± 8.73 [1–38]	-	9.87 ± 8.85 [1–38]	-
Mean back pain intensity (past 4 weeks), *1-10 NRS*	4.99 ± 1.56 [2–8]	-	4.85 ± 1.52 [2–8]	-
Maximum back pain intensity (past 4 weeks), *1-10 NRS*	7.48 ± 1.22 [5–10]	-	7.44 ± 1.23 [5–10]	-
Current back pain intensity, *1-10 NRS*	3.38 ± 1.98 [0–8]	-	3.25 ± 1.90 [0–7]	-
Pain grading[1], *n (%)*				
Grade I (low pain intensity, low disability)	23 (37.1)	-	22 (36.7)	-
Grade II (high pain intensity, low disability)	27 (43.5)	-	26 (43.3)	-
Grade III (high pain intensity and disability, moderately limiting)	8 (12.9)	-	8 (13.3)	-
Grade IV (high pain intensity and disability, severely limiting)	4 (6.4)	-	4 (6.7)	-
Regular medication, *n (%)*				
Antidepressants	2 (3.2)	-	2 (3.3)	-
Non-opioid analgesics	1 (1.6)	1(1.6)[3]	1 (1.7)	1(1.7)[3]
Pregabalin	1 (1.6)	-	1 (1.7)	-
Others[2]	10(16.1)	10 (16.4)	10(16.7)	10 (17.0)

CBP, patients with chronic back pain; HC, healthy volunteers; M, mean; SD, standard deviation; NRS, numeric rating scale. [1]Pain grading according to Von Korff et al., 1992. [2]Other medication includes antipsychotics, anti-diabetics, or antihistamines, non-steroidal anti-inflammatory drugs (NSAID), HIV medication, levothyroxine, asthma medication, statins, beta-blockers, calcium channel blockers, bronchodilators, COX-2-, angiotensin-converting-enzyme-(ACE)-, or proton-pump-inhibitors, and angiotensin-II-type-1 (AT1) receptor antagonists. No patient with CBP was treated with NSAID, benzodiazepines, or opioids (<100 mg morphine equivalent/day in line with the inclusion criteria). [3]ASS (daily dose: 100 mg). Please note that on study day 2, n = 2 patients with CBP and n = 2 HCs were additionally ruled out of data analyses due to technical issues with the experimental heat pain stimulator or mean pain intensity ratings <VAS 30 during reinstatement manipulation.

2.2. Experimental design

As described in Schlitt et al. (2021), the experimental design comprised investigations on two consecutive study days (study day 1 and study day 2). On study day 1 (~2.5h), participants were first briefed on the upcoming procedure and completed questionnaires that captured their demographic properties and information about their levels of anxiety, depression, and stress, as well as pain-related psychological characteristics (see *2.5.2. Questionnaires as subjective measures*). Thereafter, individual thermal heat pain (see *2.4 Stimuli*) thresholds were assessed at the left volar forearm (location of stimulus application, ~12cm proximally from the wrist), followed by an established calibration procedure (Forkmann et al., 2013) at the same location to identify temperature levels that reached pain intensity scores of 70 on the aforementioned 0-100 VAS (see *2.5.1 Behavioral measures)*. In preparation for the following experimental task (see *2.3. Classical differential conditioning paradigm*), two electrodes were then append to the thenar and hypothenar imminences of the non-dominant hand to record the participants' skin conductance responses during the experimental task (for details, see *2.5.2 Physiological measures*). Subsequently, the participants' arousal and pain-related fear levels were assessed on the 0–100 VAS (arousal: "How tense are you right now?"; anchors: 0 = "not tense at all" | 100 = "very tense"; pain-related fear: "How fearful are you regarding the upcoming pain stimulus?"; anchors: 0 = "not fearful at all" | 100 = "extremely fearful"). Afterwards, the instructions for the experiment were presented and participants could start the first part of the experiment (i.e., habituation phase, acquisition training, and extinction training) whenever they felt ready. Approximately 24 hours later, participants returned to the lab (study day 2, ~1h) to complete the second part of the experiment (i.e., treatment phase, recall test, reinstatement manipulation and test).

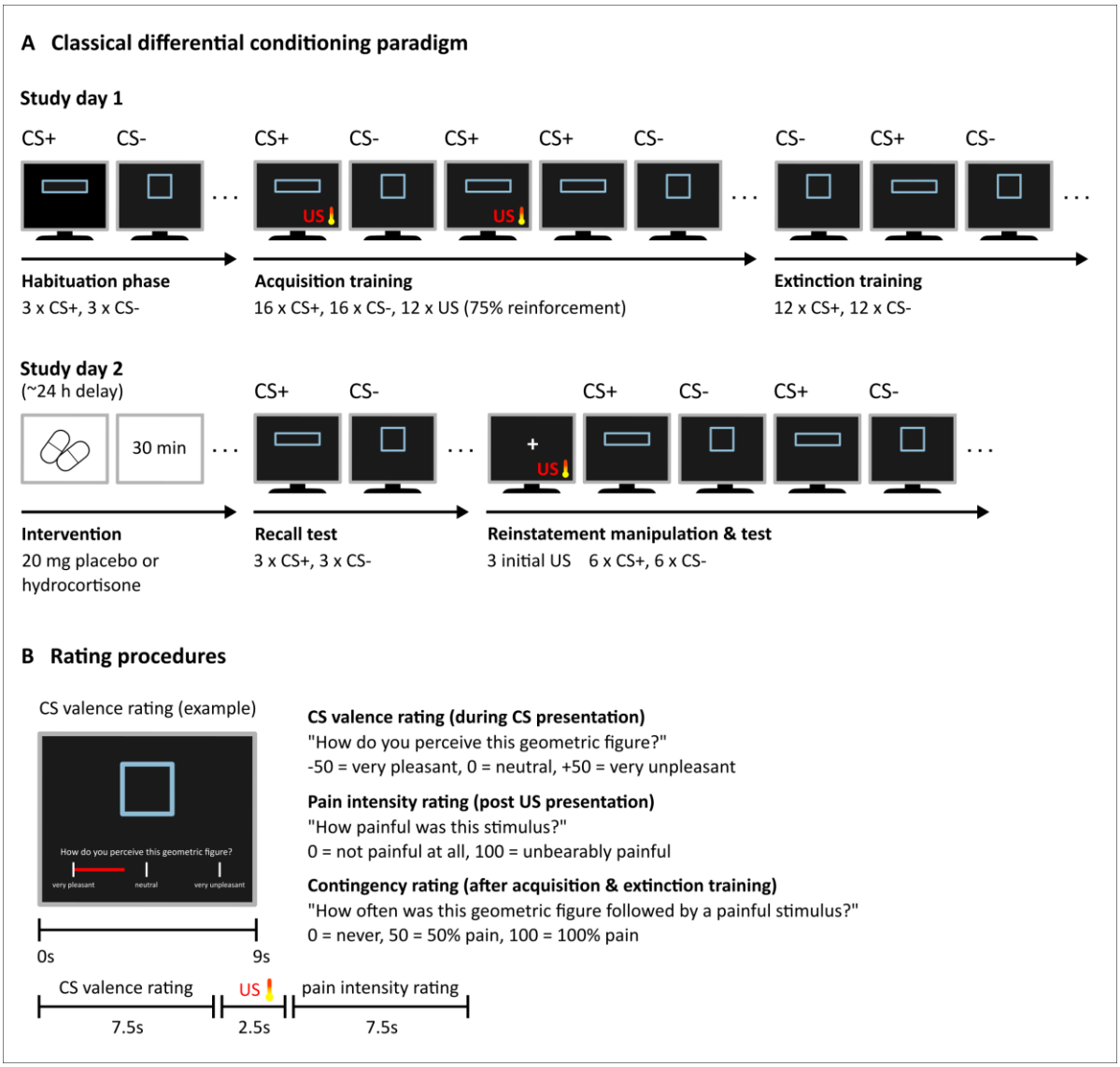

Figure 1. Experimental design. (A) The experimental classical differential conditioning paradigm was separated in two consecutive days (study day 1 and 2) and comprised 7 different experimental phases in total. On study day 1, participants completed the habituation phase (3 x CS+/CS-; valence ratings during each CS presentation), the acquisition training (16 x CS+/CS-; 12 US with a partial reinforcement rate of 75%; valence ratings during each 4th CS+/CS- presentation; pain intensity ratings after each 4th US presentation, respectively), and the extinction training (12 x CS+/CS-; valence ratings during each 4th CS+/CS- presentation). On study day 2 (~24 hours later), participants completed the second part of the experimental paradigm consisting of the (pharmacological) intervention phase, where participants were randomized to receive either 20 mg of hydrocortisone or an inactive placebo. ~30 minutes later participants continued with the recall test (3 x CS+/CS-; valence ratings during the first and third CS+/CS- presentation), which was followed by the reinstatement manipulation (3 unannounced US; pain intensity ratings after each US), and the reinstatement test phase (6 x CS+/CS-, valence ratings after the first, third, and fifth CS+/CS- presentation). (B) Rating procedures and timing: CS presentation: 9 seconds, US: 2.5 seconds; CS+-US overlap: one second; VAS presentation: 7.5 seconds (valence/pain intensity ratings), 15 seconds (contingency ratings); inter-trial interval: jittered between 6 – 11 seconds. CS, conditioned stimuli; US, unconditioned stimuli; VAS, visual analogue scale; h, hours; mg, milligrams; s, seconds. Please note that this figure is adapted based on Schlitt et al. (2021).

2.3. Classical differential conditioning paradigm and its different phases

In order to investigate pain-related learning processes in patients with CBP and pain-free HCs, and, furthermore, the influence of stress on extinction retrieval and the relapse of the original acquisition memory in both experimental groups, a classical differential conditioning paradigm was used. It was previously established in the research group by Schmidt et al. (2020) and adapted to the purposes of this study as already described in Schlitt et al. (2021) including seven experimental phases, i.e., (i) the habituation phase, (ii) acquisition training, and (iii) extinction training on study day 1, as well as (iv) the (pharmacological) intervention, (v) recall test, (vi) reinstatement manipulation, and (vii) reinstatement test on study day 2 (for details see *Figure 1. Experimental design*).

2.3.1. Habituation phase

The *habituation phase* had two main functions: (i) Participants had the opportunity to become familiar with the geometric figures and the rating procedures. (ii) Valence ratings recorded in the habituation phase served as a baseline to capture possible changes in the transition to the acquisition training. In total, only 6 CS (3 CS+, 3 CS-) were presented. Participants were asked to rate the valence of each visual stimuli after each CS presentation (for details on valence as an outcome measure, please see *2.5.1 Behavioral measures*).

2.3.2. Acquisition training

Pain-related differential learning and its temporal dynamics were investigated in the following *acquisition training*. In the preceding instruction, a possible association between the CS and the US was mentioned. However, specific CS-US-contingencies, or differences between experimental phases were not. In total, 32 CS were presented (16 CS+, 16 CS-) during the acquisition training. While 12 CS+ were presented for 8 sec prior to the application of an US (delay conditioning with a 75% reinforcement rate, 1 sec overlap), CS- were never followed by an US. The decision on a 75% partial reinforcement rate was made to elongate the extinction learning process as described in Lonsdorf et al. (2017). Participants' valence

ratings were collected at every fourth CS exposition (four valence ratings for each CS in total). This way, individual learning rates could be captured. To further track the development of the participants' pain perception, pain intensity ratings were collected after every fourth US application (three pain intensity ratings in total). After the acquisition training, the participants' contingency awareness for the CS-US pairings was assessed (for details, please refer to *2.5.1. Behavioral measures*).

2.3.3. Extinction training

Subsequently, extinction learning processes of both experimental groups were examined. Without further instructions, 24 CS (12 CS+, 12 CS-) but no US were presented during the *extinction training*. As before, participants were asked to rate the valence of the geometric figures at every fourth CS+/CS- exposition (three valence ratings for each CS in total) and indicated the prevalence of the CS-US coupling at the end of the phase, which at the same time marked the end of study day 1.

2.3.4. Pharmacological intervention

Approximately 24 h later, participants returned to the lab and received either 20 mg of hydrocortisone or an inert (placebo) pill in a double-blind fashion. Within the next 30 min (delay/latency between administration and onset of the pharmacological effect), participants completed the questionnaire battery of study day 2.

2.3.5. Recall test

During the recall test phase, the effects of pharmacologically induced stress/hydrocortisone on the strength and dominance of previously developed co-existing memory traces - they will be called acquisition and extinction memory here - were examined. Therefore, participants were re-exposed to both CS of study day 1 by presenting 6 CS (3 CS+, 3CS-) but no US during the *recall test*. CS valence ratings were collected during the first and third CS presentation, respectively. Before the experimental test session, participants were told that

the experimental task will be comparable to the one of study day 1. No specific instructions about the presence or absence of the CS and US were provided on study day 2.

2.3.6. Reinstatement manipulation

In order to initiate a reinstatement effect, i.e. the reactivation of former acquired fear memory/CRs that have been developed during acquisition training (= return of fear, ROF), participants were re-exposed to the original US as described in Lonsdorf et al., 2017. Participants thus received three single, unannounced US (2.5 sec each; same individual temperature levels as calibrated on study day 1) during the subsequent *reinstatement manipulation* phase. Pain intensity ratings were collected after each US presentation.

2.3.7. Reinstatement test

In the final *reinstatement test*, possible reinstatement effects (i.e., return of fear (ROF) after the presentation of unannounced US) were supposed to be captured. For this purpose, 12 CS (6 CS+, 6 CS-) but no US were presented subsequent to the previous reinstatement manipulation phase. Participants provided valence ratings after each first, third and fifth CS presentation, respectively.

A detailed visualization of the classical differential conditioning paradigm that was used within this study can be found in *Figure 1. Experimental design*.

2.4. Stimuli

2.4.1. Conditioned and unconditioned stimuli

Participants were presented two geometric figures that served as conditioned stimuli (CS) and that indicated either the occurrence (CS+) or omission (CS-) of a painful heat pain stimulus (unconditioned stimulus, US) during the acquisition training. Geometrical figures were randomly chosen out of a pool of three different geometric figures (i.e., rhombus, square and rectangle).

2.4.2. Stimuli presentation

The presentation of the visual (CS, VAS) and thermal stimuli (US), and, the collection of behavioral data was accomplished by the *Presentation* software (Neurobehavioral Systems, Inc., Berkeley, USA, Version 18.0, https://www.neurobs.com). Geometrical figures (color: RBG code 142, 180, 227) were designed with softened edges and presented on a black background via a computer screen for 9 sec each (square: visual angle = 4.99 × 4.99°, rectangle: visual angle = 8.3 × 3.14°, rhombus: visual angle = 7.38 × 5.36°). The presentation of the CS types was organized in a pseudorandomized order, meaning that the same CS was never presented more than two times after each other. The intertrial interval was jittered from 6–11 seconds.

Thermal heat pain stimuli (US, duration: 2.5 s) were applied using the advanced PATHWAY CHEPS (Contact Heat-Evoked Potential Stimulator, diameter: 27 mm) via a Pathway System (both Medoc, Israel). Temperatures were set to 35°C as a baseline, while heating and cooling rates were set to their maxima (70°C/s and 40°C/s respectively).

2.5. Outcome measures

The following sections will explain the outcome measures that were used in this study.

2.5.1. Behavioral measures

Various behavioral measures were collected in this study to thoroughly investigate the behavioral processes of patients with CBP and HCs that underlie pain-related learning within a classical differential conditioning paradigm.

Valence. Considering the evidence from the literature suggesting an influence of the emotional and affective components of pain on learning processes and chronification, this study included the collection of subsequent self-reported *valence ratings* across and throughout the different experimental phases on a -50 to +50 VAS ("How do you perceive this geometric figure?"; anchors: -50 = "very pleasant" | 0 = "neutral" | +50 = "very unpleasant"). Thereby, the temporal dynamics of pain-related emotional learning could be examined. Please note, that negative VAS scores indicate positive CS valence, while positive VAS scores indicate negative CS valence.

Contingency. In addition, the cognitive aspect of pain-related learning was investigated by assessing the participants' contingency awareness of the CS-US coupling via *contingency ratings* that were provided after the acquisition and extinction training for each CS type ("How often was this geometric figure followed by a painful stimulus?"; anchors: 0 = "never" | 50 = "50% pain" | 100 = "100% pain").

Pain intensity. *Pain intensity ratings* were collected to (i) verify that the selected temperatures yielded the intended pain intensity level of ~70 on the 0-100 VAS over the course of the experiment, and (ii) to ensure comparable pain perception between experimental groups ("How painful was this stimulus?"; anchors: 0 = "not painful at all" | 100 = "unbearably painful").

The VAS was presented either underneath the CS (valence and contingency ratings) or in the center of the screen (pain intensity ratings). The rating scale was always located at a random

starting point between 25 and 75 on the VAS and participants had to submit their ratings within 7.5 seconds (except for contingency ratings: within 15 seconds). In case participants did not submit their ratings in time, those ratings were excluded from the final data analysis.

2.5.2. Physiological measure

Complementary to the self-reported valence ratings, *skin conductance responses (SCRs)* were constantly assessed across all phases as a *physiological measure*. Therefore, radio translucent dry electrodes (EL 509, BIOPAC Systems Inc., Goleta, USA) that were stuck to the thenar and hypothenar eminences of the participants' non-dominant hand upon a conductive electrode cream (SYNAPSE, Kustomer Kinetics) were connected to a BIOPAC MP150 device (BIOPAC Systems, Inc.) with a constant voltage system (0.5 V). Recordings were performed using the *AcqKnowledge* software (version 5.0.2) with a sampling rate of 2 kHz. In order to tag the precise onset of an event, external triggers were additionally recorded. As an indicator for emotional arousal through sympathetic nervous system activation by affective or salient stimuli (Dawson et al., 2007; Wallin, 1981) and therefore, physiological conditioned responses (CRs), SCRs are considered an exquisite measure for investigating reinstatement effects in human fear conditioning (Lonsdorf et al., 2017).

2.5.3. Neuroendocrine measure

Since this study included a placebo-controlled hydrocortisone treatment (study day 2), *saliva samples* were collected as a *neuroendocrine measure* (Kirschbaum and Hellhammer, 1994) to compare free salivary cortisol levels in both treatment conditions (hydrocortisone, placebo) within each experimental group (patients with CBP, HCs). Hence, participants provided saliva samples at distinct predetermined time points by chewing Salivette sampling devices (Sarstedt, Nümbrecht, Germany) for 60 seconds. Samples were collected (1) prior to and (2) post experiment on study day 1, (3) prior to the pharmacological intervention, (4) 30 min post treatment, and (5) immediately after the experiment on study day 2. The samples were collected and intermediately stored at the lab at 5°C for up to 7 days. Afterwards, they

were centrifuged and kept at -20°C until the biochemical analysis. Due to sample loss, data of n=3 HCs and n=3 patients with CBP were missing for data analysis. The salivary samples were further processed in a cooperating lab at the Institute of Medical Psychology and Behavioral Immunobiology at the University Clinic Essen (Germany). There, free salivary cortisol concentrations were determined by enzyme-linked immunosorbent assay (Cortisol Saliva ELISA, IBL International, Hamburg, Germany) as specified by the manufacturer with inter- and intra-assay coefficients of variation <10%. Cross-reactivity of the anti-cortisol antibody with other relevant steroids was 8.5% (11-deoxycortisol), 2.6% (cortisone), 1.0% (corticosterone), and <0.1% (estrone, estradiol, estriol, progesterone, testosterone). As a standard procedure to decrease error variance caused by imprecisions of the intra-assay, all samples of each participant were processed at the same run.

2.5.4. Questionnaires as subjective measures

To characterize the patient sample and to investigate the modulation of pain-related learning processes by different psychological variables, German versions of the following questionnaires were assessed: (1) State-Trait-Anxiety-Depression-Inventory (Laux et al., 2013); (2) Depression Anxiety Stress Scales (Nilges and Essau, 2015); (3) Center for Epidemiological Studies-Depression Scale: ADS-K (Hautzinger and Bailer, 1993); (4) Pain Catastrophizing Scale: PCS (Lautenbacher et al., 2009); (5) Pain Anxiety Symptom Scale: PASS-D (Walter et al., 2002); (6) Trier Inventory of Chronic Stress: TICS (Schulz et al., 2004); (7) Questionnaire for Experiences of Attention Deficits (Zimmermann et al., 1991); (8) Perceived Stress Questionnaire: PSQ20 (Fliege et al., 2001). All questionnaires were analyzed using their respective manuals. Different scores calculated for both experimental groups and the results of the comparison between both groups are listed in *Table 3. Pain-related, fear, arousal, and self-report measures.* Since recent findings revealed a modulating role of psychological trait and state variables on pain perception, chronification, and learning (Nees and Becker, 2018), the scores of the questionnaires were later tested as potential covariates in further, explorative analyses.

2.6. Statistical analyses

Data preprocessing and statistical analyses were performed using the software *R Studio* (RStudio Team, 2016, version 1.4.1103).

Overall, two different strategies for analyzing the behavioral and SCR data from study day 1 and study day 2 were applied: For the analysis of the study day 1 data, the factor *time* was implemented as a continuous factor into the model since the temporal dynamics of acquisition and extinction learning of pain-related threat and safety associations (i.e., the development / slopes of the CS valence ratings and SCRs) in patients with CBP and HCs were investigated. In the following, type III *analyses of variance (ANOVA)* according to Satterthwaite's method were calculated on separate *linear mixed models (LMMs)* for acquisition and extinction training, and β-values from separate LMM analyses were reported equivalent to Schlitt et al. (2021), if the initial ANOVA revealed statistically significant *main effects (MEs)* or *interactions (IAs).* For the ANOVAs, *partial eta-square* (η_p^2) is reported as effect size, while *Cohen's d* was calculated for LMMs. The analysis of the behavioral and SCR data from study day 2 focused on initial responses (i) during the recall (i.e., retrieval of extinction or acquisition memory) and (ii) the reinstatement test (i.e., extinction efficacy), and (iii) the influence of hydrocortisone on these processes in both groups. Thus, CS valence ratings and SCRs assessed (i) at the end of the extinction training and at the beginning of the recall test as well as (ii) at the end of the recall test and at the beginning of the reinstatement test were directly compared. Hence, the factor *time* was implemented as a discrete variable and ANOVAs were performed on separate LMMs. Significant results were followed by Bonferroni-Holm adjusted post-hoc tests and *Cohen's d* was further calculated as effect size. Therefore, the *R* package *EMAtools* (Kleiman, 2017) was used.

Further, a 2-step analysis strategy was chosen for the analysis of both study day 1 and study day 2 data in order to investigate MEs and IAs for the factors *group* (HC, CBP), *time*, and, explicitly on study day 2, *treatment condition* (hydrocortisone, placebo). Thus, differential learning, i.e., the change in differences between CRs to the CS+ and to the CS- in valence ratings and SCRs (ΔCS valence = valence CS+ - valence CS-; ΔSCR = SCR amplitudes on

CS+ - SCR amplitudes on CS-) were investigated fist. Further, in non-differential analyses separately for both *CS types* (CS+, CS-) it was examined, whether the differences or changes were based on altered CRs to the CS+, the CS-, or both CS.

To identify the best fitted model, different models were compared before any analysis using the *anova* function of the *R* package *stats* (Chambers et al., 1992) for calculating the chi-square (χ^2) between each model, i.e., explained variance (*maximum likelihood method*). Here, it was tested, whether allowing for inter-individual differences in baseline valence ratings for each participant (including a random intercept per participant) and varying the factors *time*, *group*, *CS type*, *treatment*, and *participants* including random effects for these factors, improved model fit, and therefore indicates a better prediction of data. Models were thus selected based on the *Akaike Information Criterion (AIC)*. Best-fitting models are listed in *Table 2 – Models used for data analysis*. MEs and IAs were considered as statistically significant when reaching *p* values < 0.05.

2.6.1. Valence ratings

To investigate changes of differential and non-differential CS valence ratings from the habituation phase through the acquisition training within and between experimental groups, mean ratings of the habituation phase were calculated and added as a baseline value. Accordingly, CS valence ratings of the extinction training phase were analyzed by implementing the last CS valence ratings of the acquisition training as baseline values. For the analysis of extinction retrieval or spontaneous recovery effects the last CS valence ratings of the extinction training (study day 1) were compared with the first CS valence ratings of the retrieval test (study day 2). The occurrence of a reinstatement effect (and therefore, extinction efficacy) was tested by comparing the last CS valence ratings of the recall test with the first CS valence rating of the reinstatement test (both study day 2).

2.6.2. Contingency ratings

Dissimilarities in contingency awareness of the CS-US pairing between groups, and changes from the acquisition to the extinction training were analyzed calculating ANOVAs on separate LMMs for the contingency ratings assessed post acquisition and extinction training.

2.6.3. Skin conductance responses

Before SCR data could be analyzed, the following preprocessing steps had to be conducted using the software *R*: SCR data were first down-sampled to 20 Hz and smoothed via a low-pass filter (cutoff frequency = 2 Hz). After the automated identification of local minima and maxima, amplitudes of stimulus-related SCRs were calculated subtracting the local minimum at the first SCR onset after stimulus onset from the maximum peak (Prokasy and Ebel, 1967).

In the context of fear and extinction learning, recent studies recommend that SCRs might be divided into different time windows in order to track and analyze different dimensions of learning (Boucsein et al., 2012; Jentsch et al., 2020). Therefore, the first-interval response (FIR; further also referred to as early conditioned SCR) is commonly associated with orienting behavior and responses to novel stimuli that usually habituate over time (Öhman, 1974, 1972), as well as associative learning processes (Jentsch et al., 2020). The second-interval response (SIR, further also referred to as late conditioned SCR) rather captures emotional reactions to CS when expecting a US (Jentsch et al., 2020; Öhman, 1972; Wolter and Lachnit, 1993). Thus, a temporal shift from early to late conditioned SCRs during acquisition learning is discussed to underlie two processes, one about the CS-US association and one about the relative CS-US timing that develop gradual but are intertwined at the same time (Jentsch et al., 2020). Therefore, FIRs to the CS were analyzed using a time window of 1-5 seconds, while SIRs were examined within a time window of 5–9.5 seconds after CS onset (Boucsein et al., 2012; Jentsch et al., 2020) at a presented CS length of 9 seconds. Unconditioned responses to the US (duration 2.5 seconds) were analyzed using a time window of 0.5–7 seconds after onset of the US. Responses below the minimum amplitude criterion of 0.1µS were scored as 0.0 µS. To decrease the skew of the amplitude distribution

and to achieve normal distribution, SCR data were additionally transformed with the natural logarithm (Ishihara and Miyata, 1980). Further, only trials without VAS ratings were included into the SCR analysis to minimize a contamination with signal changes that were due to movement artifacts during the rating procedure. Lastly, an outlier detection was performed defining SCRs that deviated >3 SDs from the individual mean as outliers. Therefore, n=2 SCRs were excluded from the analysis of study day 1 SCR data.

Please note that since different aspects of learning were investigated during study day 1 and study day 2, two different strategies for SCR data analysis were followed. (1) To investigate the temporal dynamics of pain-related acquisition and extinction learning in patients with CBP as compared to HCs (study day 1), SCRs between valence ratings were pooled for 3 continuous trials such that four pooled SCRs for the analysis of the acquisition training and three pooled SCRs for the analysis of the extinction training phase were obtained. According to the analyses of CS valence ratings, analyses of the temporal changes in SCR amplitudes were performed separately for both experimental phases. (2) To test for distinct extinction retrieval or spontaneous recovery effects, SCRs of the 11^{th} (i.e., second last) extinction training trial and the second recall test trial were compared between experimental and treatment groups. Note, that the last extinction training and the first recall test trials included CS valence ratings and were not analyzed due to potential movement-induced artifacts that could occur during rating procedures. Reinstatement effects (i.e., extinction efficacy) were examined comparing SCRs of the 2^{nd} recall and reinstatement test trials. Again, please note that the last recall test and the first reinstatement test trials included CS valence ratings.

Due to technical issues during the recording of the SCRs, 3 participants (n=2 patients with CBP, n=1 HC) were ruled out from the SCR data analysis of study day 1, while n=4 HCs had to be excluded from study day 2 SCR data analysis additionally to the former mentioned exclusion criteria (*2.1.4. Final sample for data analysis*). Therefore, SCR data analysis of study day 1 was based on n=60 patients with CBP and n=60 HCs, while the SCR data of study day 2 comprised n=58 patients with CBP and n=55 HCs.

2.6.4. Person-, disease- and pain-related affective variables as modulators for pain-related learning

For patients with CBP, pain-related acquisition and extinction learning as well as extinction retrieval and/or reinstatement effects were tested to be modulated by clinical pain duration. Hence, pain duration (in years) was incorporated as a covariate of interest (controlling for age). Beyond that, exploratory analyses were conducted on pain- and person-related affective measures in order to test the modulatory role of these covariates on CS valence ratings, SCRs, or contingency awareness. Those included state/trait anxiety and depression (STADI), pain anxiety (PASS), pain catastrophizing (PCS), arousal, and pain-related fear (both through VAS rating). Since scores of the PCS and PASS were found to be positively correlated, both scores were included as a covariate of interest into the respective models while controlling for the other one (i.e., covariate of non interest).

Table 2. Models used for data analysis.

Phase	**Measure**	**Fixed effects**	**Random effects and R code**	**AIC**
Acquisition training	Δ CS valence	*time* × *group*	Random intercept: *participants*, Random slopes: *participants*, *time* Δ CS valence ~ time*group + (1 + time | participants)	*Δ AIC*: -70.6 $p < 0.001$
	CS valence (non-differential)	*time* × *group* × *CS type*	Random intercept: *participants*, Random slopes: *participants*, *time*, *CS type* CS valence ~ time*group*CS type + (1 + time, CS type | participants)	*Δ AIC*: -685.5 $p < 0.001$
	Δ CS-related SCR	*time* × *group*	Random intercept: *participants*, Random slopes: *participants* Δ CS-SCR ~ time*group + (1 | participants)	*
	CS-related SCR (non-differential)	*time* × *group* × *CS type*	Random intercept: *participants*, Random slopes: *participants*, *time*, *group*, *CS type* CS-SCR ~ time*group*CS type + (1 + time, group, CS type | participants)	*Δ AIC*: -89.4 $p < 0.001$
	US-related SCR	*time* × *group*	Random intercept: *participants*, Random slopes: *participants*, *time* US-SCR ~ time*group + (1 + time | participants)	*Δ AIC*: -70.9 $p < 0.001$
Extinction training	Δ CS valence	*time* × *group*	Random intercept: *participants*, Random slopes: *participants*, *time* Δ CS valence ~ time*group + (1 + time | participants)	*Δ AIC*: -75.5 $p < 0.001$
	CS valence (non-differential)	*time* × *group* × *CS type*	Random intercept: *participants*, Random slopes: *participants*, *time*, *CS type* CS valence ~ time*group*CS type + (1 + time, CS type | participants)	*Δ AIC*: -629.6 $p < 0.001$
	Δ CS-related SCR	*time* × *group*	Random intercept: *participants*, Random slopes: *participants* Δ CS-SCR ~ time*group + (1 | participants)	*
	CS-related SCR (non-differential)	*time* × *group* × *CS type*	Random intercept: *participants*, Random slopes: *participants*, *CS type* CS-SCR ~ time*group*CS type + (1 + CS type | participants)	*Δ AIC*: -49.6 $p < 0.001$
Contingency	Contingency	*phase* × *group* × *CS type*	Random intercept: *participants*, Random slopes: *participants*, *group*, *CS type* Contingency ~ phase*group*CS type + (1 + group, CS type | participants)	*Δ AIC*: -34.7 $p < 0.001$

Recall test	Δ CS valence	*time* × *group* × *treatment*	Random intercept: *participants*, Random slopes: *participants* Δ CS valence ~ time*group*treatment + (1 | participants)	*
	CS valence (non-differential)	*time* × *group* × *CS type* × *treatment*	Random intercept: *participants*, Random slopes: *participants*, *time* CS valence ~ time*group*CS type*treatment + (1 + time | participants)	*Δ AIC*: -348.2 $p < 0.001$
	Δ CS-related SCR	*time* × *group* × *treatment*	Random intercept: *participants*, Random slopes: *participants* Δ CS-SCR ~ time*group*treatment + (1 | participants)	*
	CS-related SCR (non-differential)	*time* × *group* × *CS type* × *treatment*	Random intercept: *participants*, Random slopes: *participants*, *time* CS-SCR ~ time*group*CS type*treatment + (1 + time | participants)	*Δ AIC*: -3.9 $p = 0.02$
Reinstatement manipulation	US-related SCR	*time* × *group* × *treatment*	Random intercept: *participants*, Random slopes: *participants* US-SCR ~ time*group*treatment + (1 | participants)	*
Reinstatement test	Δ CS valence	*time* × *group* × *treatment*	Random intercept: *participants*, Random slopes: *participants* Δ CS valence ~ time*group*treatment + (1 | participants)	*
	CS valence (non-differential)	*time* × *group* × *CS type* × *treatment*	Random intercept: *participants*, Random slopes: *participants*, *CS type* CS valence ~ time*group*CS type*treatment + (1 + CS type | participants)	*Δ AIC*: -944.2 $p < 0.001$
	Δ CS-related SCR	*time* × *group* × *treatment*	Random intercept: *participants*, Random slopes: *participants* Δ CS-SCR ~ time*group*treatment + (1 | participants)	*
	CS-related SCR (non-differential)	*time* × *group* × *CS type* × *treatment*	Random intercept: *participants*, Random slopes: *participants*, *time* CS-SCR ~ time*group*CS type*treatment + (1 + time | participants)	*Δ AIC*: -19.2 $p = 0.02$

CS, conditioned stimulus; US, unconditioned stimulus; SCR, Skin conductance responses; AIC, Akaike Information Criterion; *Number observations was too low.

3. Results

3.1. Self-report and pain-related measures

Patients with CBP and HCs differed significantly in all assessed psychological state and trait, and pain-related cognitive variables. Despite this, scores of most of the patients were still in a normal range across all psychological and pain-related self-report questionnaires. Further, patients with CBP and HCs showed comparable heat pain thresholds, calibrated temperature levels for the US, arousal ratings, pain-related fear ratings or pain intensity ratings during acquisition training. Further, pain intensity ratings assessed during the reinstatement manipulation phase (study day 2) did not differ as well and were still moderate to high in both experimental groups and treatment conditions. For detailed information about scores and statistics please see *Table 3 – Pain-related, fear, arousal, and self-report measures*.

3.2. Habituation Phase

Neither differential, nor non-differential CS valence ratings of the habituation phase exhibited significant differences (all $p > 0.05$) for the different CS types (CS+ vs. CS-) within or between experimental groups (patients with CBP vs. HCs), which further ensured no affective connotation of the visual stimuli prior to conditioning. CS valence ratings of all experimental phases are visualized in Figures 2 (differential) and 3 (non-differential).

3.3. Acquisition Training

Valence ratings. An initial *time* × *group* interaction (IA; $F(1,120.86) = 4.69$, $p = 0.03$, $\eta_p^2 = 0.04$) indicated statistically significantly different changes in the development of ΔCS valence ratings from the habituation through the acquisition training between patients with CBP and HCs. However, statistically significant increases in ΔCS valences from the habituation through the acquisition training in both patients and HCs (*Figure 2. Differential CS valence ratings)* indicate successful differential learning in both experimental groups (patients with CBP: $\beta = 3.45 \pm 0.84$; $t(121.98) = 4.11$, $p < 0.001$, $d = 0.74$; HCs: $\beta = 6.02 \pm$

0.84; $t(119.75) = 7.15$, $p < 0.001$, $d = 1.31$), even though attenuated in the patient group ($\Delta\beta = -2.57 \pm 1.19$; $t(120.86) = -2.17$, $p = 0.03$, $d = -0.39$). Analyses of non-differential CS valence ratings also revealed a statistically significant *time* × *group* × *CS type* IA ($F(1,816.10) = 10.72$, $p = 0.001$, $\eta_p^2 = 0.01$). In detail, CS+ valence ratings significantly increased in negative valence (patients with CBP: $\beta = 2.54 \pm 0.45$; $t(315.43) = 5.62$, $p < 0.001$, $d = 0.63$; HCs: $\beta = 3.81 \pm 0.45$; $t(312.62) = 8.37$, $p < 0.001$, $d = 0.95$), while CS- valence ratings significantly increased in positive valence over the course of the acquisition training in both experimental groups (patients with CBP: $\beta = -0.88 \pm 0.45$; $t(313.07) = -1.94$, $p = 0.05$, $d = -0.22$; HCs: $\beta = -2.27 \pm 0.45$; $t(307.97) = -5.02$, $p < 0.001$, $d = -0.57$). As compared to HCs, in patients with CBP both effects were weaker (IA *time x group*, CS+: $\Delta\beta = -1.26 \pm 0.64$; $t(314.02) = -1.96$, $p = 0.05$, $d = -0.22$; CS-: $\Delta\beta = 1.40 \pm 0.64$; $t(310.50) = 2.18$, $p = 0.03$, $d = 0.25$).

Skin conductance responses. In line with the beforehand mentioned association of first-interval responses (FIRs) with orienting behavior and novel stimuli and their usually observed habituation over time (Öhman, 1974, 1972), early conditioned SCRs to the CS+ and CS- significantly decreased (*Figure 5. Non-differential early conditioned skin conductance responses (first interval responses, FIR)*) during acquisition training in both experimental groups (ME *time*; $F(1,112.89) = 29.00$, $p < 0.001$, $\eta_p^2 = 0.20$). No significant IAs within or between experimental groups were found (all $p > 0.05$). The analysis of the second-interval responses (SIRs) did not reveal any significant main effects (MEs) or IAs within or between both experimental groups (all $p > 0.05$).

Contingency ratings after acquisition training. Both experimental groups revealed statistically significant differences in their contingency ratings for the CS+ compared to the CS- (*Figure 8. Contingency awareness*), indicating successful differential learning and a general accuracy in the CS-US contingency awareness after the acquisition training (ME *CS type*; $F(1,119.58) = 226.60$, $p < 0.001$, $\eta_p^2 = 0.73$).

Modulatory role of pain-related and affective measures on the acquisition training.

According to a *time* × *CS type* × *group* × *state anxiety* IA ($F(1,806.57) = 6.71$, $p = 0.01$, $\eta_p^2 = 0.01$), state anxiety was found to modulate pain-related acquisition learning differently in patients with CBP and HCs. While higher state anxiety scores obtained by the STADI questionnaire were associated with steeper increases in CS+ valence ratings (i.e., enhanced threat learning) in patients with CBP ($\beta = 0.19 \pm 0.08$; $t(919.53) = 2.45$, $p = 0.01$, $d = 0.26$), HCs with higher state anxiety scores revealed steeper decreases in CS- valence ratings (i.e., enhanced safety learning) ($\beta = -0.37 \pm 0.15$; $t(306.71) = -2.49$, $p = 0.01$, $d = 0.28$).

To analyze the modulatory role of pain anxiety (assessed through the Pain Anxiety Symptom Scale, PASS-D) as a covariate of interest, pain catastrophizing (assessed through the Pain Catastrophizing Scale, PCS) was included as a covariate of non-interest into the model and vice versa. Statistically significant *time* × *group* × *pain anxiety* and *time* × *group* × *pain catastrophizing* IAs (pain anxiety: $F(1,115.60) = 6.27$, $p = 0.01$, $\eta_p^2 = 0.05$; pain catastrophizing: $F(1,637.56) = 4.75$, $p = 0.03$, $\eta_p^2 = 0.01$) further revealed that in patients with CBP only, higher pain anxiety and pain catastrophizing scores were associated with steeper increases in CS+ valence ratings during the acquisition training (pain anxiety: $\beta = 0.09 \pm 0.04$; $t(315.92) = 2.49$, $p = 0.01$, $d = 0.28$; pain catastrophizing: $\beta = 0.13 \pm 0.05$; $t(923.21) = 2.92$, $p = 0.004$, $d = 0.31$). Thus, patients with CBP who tend to be more anxious and catastrophizing with regard to pain seem to have a quicker pain-related acquisition learning.

Arousal modulated merely CS+ valence ratings in HCs. An initial statistically significant *time* × *CS type* × *group* × *arousal* IA ($F(1,610.11) = 10.91$, $p = 0.001$, $\eta_p^2 = 0.02$), revealed that higher arousal ratings were related to steeper increases in CS+ valences during acquisition training ($\beta = 0.11 \pm 0.03$; $t(195.37) = 3.51$, $p < 0.001$, $d = 0.50$). This indicates elevated threat learning in more aroused HCs.

Modulatory role of pain duration on the acquisition training.

In an exploratory analysis, patients that reported longer pain duration revealed a trend towards less increase in their CS+ valence ratings during acquisition training (IA *time* × *pain*

duration: β = -0.09 ± 0.05; *t*(179.08) = -1.84, *p* = 0.07, *d* = -0.28). Further, a statistically significant *time* × *CS type* × *pain duration* IA (*F*(1,122.46) = 5.16, *p* = 0.03, η_p^2 = 0.04) revealed that patients with longer pain duration, provided significantly lower contingency ratings for the CS+/US coupling during the acquisition training (β = -1.53 ± 0.32; *t*(97.25) = -4.79, *p* < 0.001, *d* = -0.97). Taken together, these exploratory findings suggest that already impaired threat learning capacities in patients with CBP, worsens with longer pain duration.

3.4. Extinction Training

Valence ratings. ΔCS valences of both experimental groups significantly decreased over the course of the extinction training indicating the general capability for differential extinction learning in both experimental groups (*ME time*: *F*(1,120.53) = 74.81, *p* < 0.001, η_p^2 = 0.38).

Further, a statistically significant *time* × *CS type* IA (*F*(1,601.00) = 159.51, *p* < 0.001, η_p^2 = 0.21) of the non-differential analysis revealed that the decrease of the ΔCS valences was driven by a change of the valence ratings of the CS+ back to more positive valences in both groups (patients with CBP: β = -4.20 ± 0.60; *t*(246.98) = -7.06, *p* < 0.001, *d* = -0.90; HCs: β = -6.02 ± 0.60; *t*(247.00) = -10.04, *p* < 0.001, *d* = -1.28). CS- valences on the other hand did not change over the course of the extinction training and maintained at a positive level (patients with CBP: β = 0.87 ± 0.60; *t*(251.47) = 1.44, *p* = 0.15, *d* = 0.18; HCs: β = 0.90 ± 0.60; *t*(244.58) = 1.50, *p* = 0.13, *d* = 0.19).

Skin conductance responses. Neither the analysis of the FIRs nor SIRs revealed any significant main effects (MEs) nor IAs within or between experimental groups (all *p* > 0.05).

Contingency ratings after extinction training. Compared with contingency ratings after the acquisition training, participants of both experimental groups provided significantly lower contingency ratings regarding the CS-US pairing for both CS types after extinction training (ME *time*: *F*(1,167.73) = 260.48, *p* < 0.001, η_p^2 = 0.34) which is another indicator for successful extinction learning. Neither phase specific nor statistically significant differences

in the changes of contingency ratings from the acquisition to extinction training were observed between both groups (all p > 0.05).

Modulatory role of pain-related and affective measures on the extinction training.

According to the acquisition training, exploratory analyses were conducted for state anxiety, pain anxiety, pain catastrophizing, and arousal. Here, only in the HCs' valence ratings, a statistically significant *time* × *CS type* × *state anxiety IA* ($F(1,593.30) = 5.56$, $p = 0.02$, $\eta_p^2 = 0.01$) revealed stronger differential extinction learning in HCs with higher state anxiety scores ($\beta = 0.56 \pm 0.23$; $t(592.93) = 2.46$, $p = 0.01$, $d = 0.20$).

Modulations of pain anxiety and catastrophizing on valence ratings of the extinction training were only found in patients with CBP. The statistically significant IAs *time* × *pain anxiety* ($F(1,593.30) = 5.56$, $p = 0.02$, $\eta_p^2 = 0.01$) and *CS type* × *pain catastrophizing* ($F(1,617.50) = 10.29$, $p = 0.001$, $\eta_p^2 = 0.02$) revealed that higher pain anxiety and catastrophizing scores were associated with enhanced extinction learning related to CS+ valence ratings (pain anxiety: $\beta = -0.15 \pm 0.05$; $t(248.13) = -3.14$, $p = 0.002$, $d = -0.40$; pain catastrophizing: $\beta = -0.17 \pm 0.07$; $t(249.26) = -2.61$, $p = 0.01$, $d = -0.33$). This might be due to the enhanced acquisition learning that was moderated by both pain anxiety and catastrophizing (i.e., *floor effect*).

Modulatory role of pain duration on the extinction training.

In the patients, a statistically significant *time* × *CS type* × *pain duration* IA ($F(1,303.19) = 8.47$, $p = 0.004$, $\eta_p^2 = 0.03$) further revealed significantly less decrease in their CS+ valence ratings with increased pain duration (IA *time* × *pain duration*: $\beta = 0.17 \pm 0.06$; $t(114.76) = 2.84$, $p = 0.005$, $d = 0.53$). For the contingency ratings, a statistically significant *time* × *CS type* × *pain duration* IA ($F(1,122.46) = 5.16$, $p = 0.03$, $\eta_p^2 = 0.04$) further revealed that pain duration negatively modulated changes in the patients' contingency ratings for the CS+ from acquisition to extinction training (i.e., less decrease in CS+ contingency ratings) as indicated by a *time* x *pain duration* IA ($\beta = 1.15 \pm 0.38$; $t(117.15) = 3.00$, $p = 0.003$, $d = 0.56$). Thus, pain duration not only seems to worsen the already impaired threat learning of patients with CBP but further negatively affects their extinction learning capabilities as well.

Table 3. Pain-related, fear, arousal, and self-report measures.

	CBP (M±SD)		HC (M±SD)		Statistics	p-Value
Heat pain-related data*						
Heat pain thresholds *(°C)*	43.8 ± 2.46		43.8 ± 1.63		W = 1809.50	0.68
Temperature US *(°C)*	47.5 ± 1.84		47.7 ± 1.48		W = 2020.00	0.52
Pain intensity ratings						
Acquisition training *(0-100 VAS)*	62.5 ± 12.8		64.4 ± 10.4		t(121) = 0.91	0.37
Reinstatement manipulation *(0-100 VAS)*	Placebo 67.59±12.08	Hydro 69.40±14.64	Placebo 69.15±11.16	Hydro 65.86±13.55	F(1,114)=1.13	0.29
Questionnaire data						
STADI						
state anxiety	18.00 ± 5.48		15.20 ± 3.02		W = 1318.50	**0.005**
state depression	18.10 ± 4.51		15.80 ± 2.88		W = 1203.00	**<0.001**
trait anxiety	21.20 ± 6.19		17.00 ± 4.37		W = 1097.50	**<0.001**
trait depression	17.80 ± 4.29		15.50 ± 3.23		W = 1255.50	**0.002**
CES-D[1]	9.68 ± 6.85		6.22 ± 5.28		W = 1265.00	**0.002**
PCS	20.40 ± 9.16		11.80 ± 10.10		W = 971.50	**<0.001**
PASS 20-D	31.90 ± 12.40		26.30 ± 14.40		t(120) = -2.30	**0.023**
DASS						
depression[2]	3.97 ± 3.85		1.30 ± 1.60		W = 947.00	**<0.001**
anxiety[3]	3.39 ± 3.07		1.11 ± 1.39		W = 970.00	**<0.001**
stress[4]	7.15 ± 4.65		2.26 ± 2.59		W = 619.50	**<0.001**
TICS	20.90 ± 8.89		16.00 ± 7.71		t(120) = -3.23	**0.002**
PSQ20	55.5 ± 18.9		43.9 ± 17.2		t(120) = -3.57	**<0.001**
FEDA	100.00 ± 13.10		107.00 ± 10.00		W = 2384.00	**0.007**

HC, healthy volunteers; CBP, patients with chronic back pain; Placebo, placebo treatment group; Hydro, hydrocortisone treatment group; STADI, State-Trait-Anxiety-Depression-Inventory; CES-D, Center for Epidemiologic Studies Depression Scale; PCS, Pain Catastrophizing Scale; PASS 20-D, Pain Anxiety Symptom Scale, German version; DASS, Depression Anxiety Stress Scales; TICS, Trier Inventory of Chronic Stress; PSQ20, Perceived Stress Questionnaire; FEDA, Questionnaire for Experiences of Attention Deficits. Please note that due to technical issues, questionnaire data (except for DASS) of n=1 HC got lost. [1] n=9 CBP patients (14.5%) scored above the cut-off of 16, [2] n=4 CBP patients (6.5%) above cut-off of 10; [3] n=11 CBP patients (17.7%) above cut-off of 6; [4] n=14 CBP patients (22.6%) above cut-off of 10.

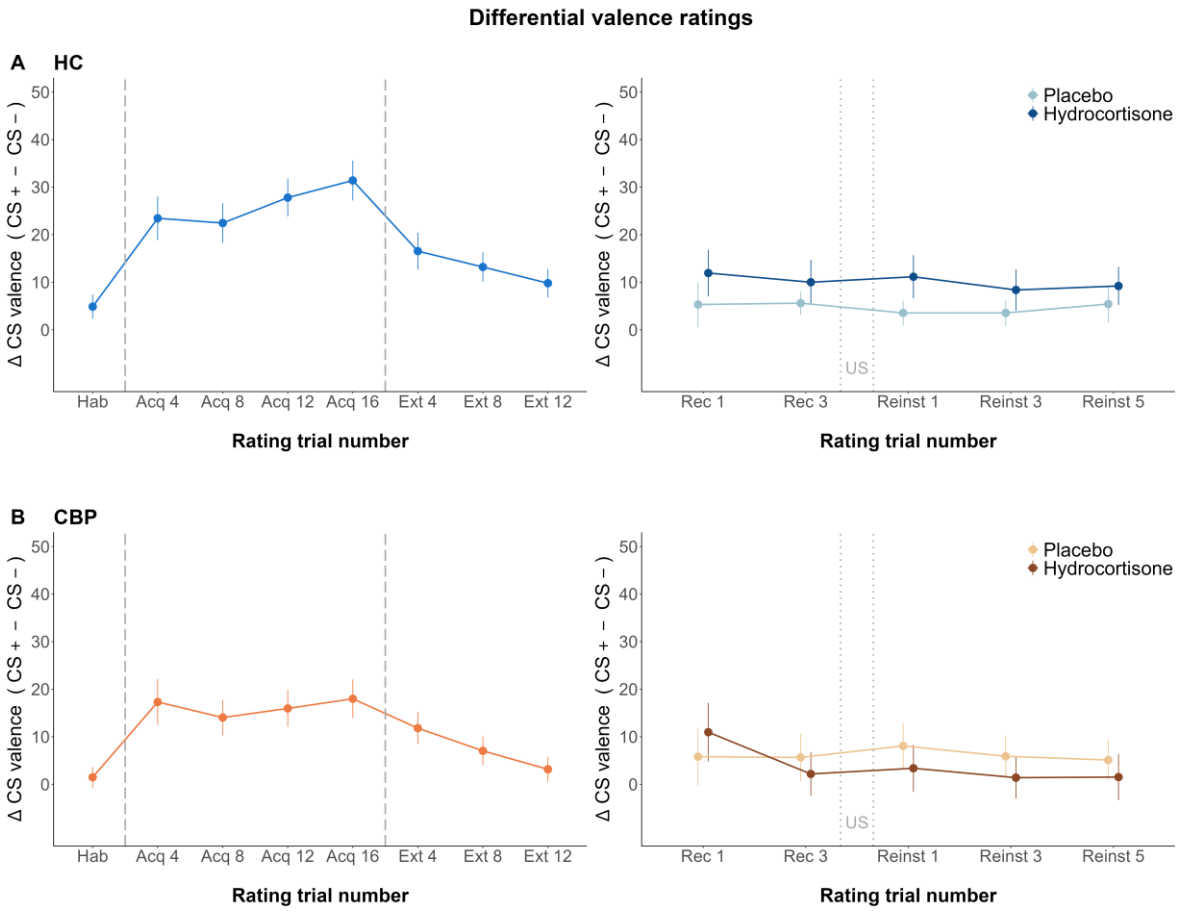

Figure 2. Differential CS valence ratings. Mean differential CS valence ratings (CS+ - CS-) ± standard error of the mean (SEM) of patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue) throughout all experimental phases. Since the hydrocortisone vs. placebo treatment was applied on study day 2, mean differential CS valence ratings are split into placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions on the right graphs. Rating trial numbers define the exact trials of the different experimental phases (Hab, habituation; Acq, acquisition training, Ext, extinction training; Rec, recall test; Reinst, reinstatement test) at which CS valence ratings were assessed. Dashed lines indicated the transition from one experimental phase to the next within study day 1, while dotted lines indicate the reinstatement manipulation phase of study day 2 at which the unconditioned stimulus (US) was applied without pairing to the CS+ in order to investigate extinction efficacy or possible reinstatement effects. CS, conditioned stimulus.

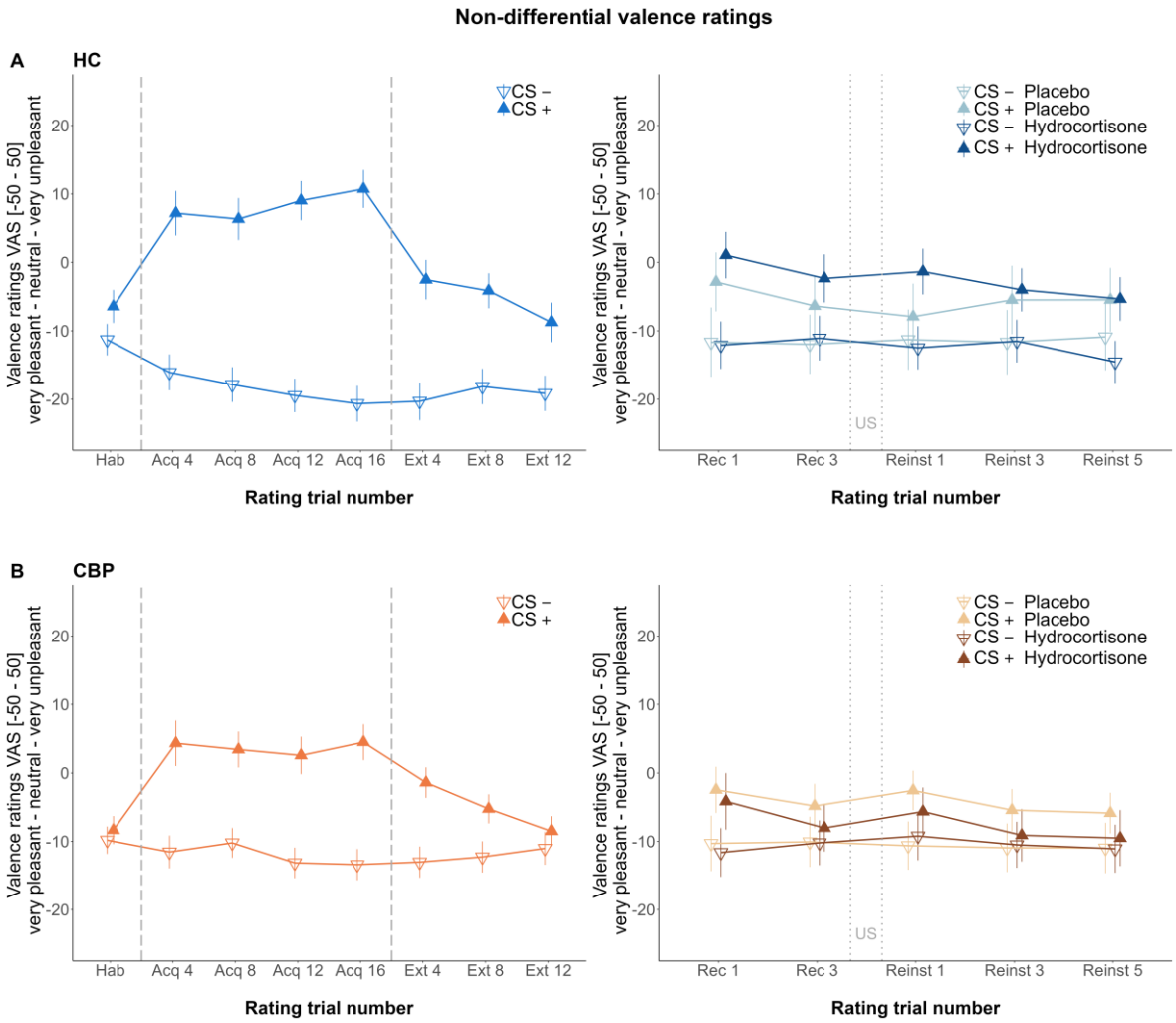

Figure 3. Non-differential CS valence ratings. Mean CS valence ratings ± standard error of the mean (SEM) of patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue) for the CS+ (filled triangles) and the CS- (unfilled, upside-down triangles) over the course of all experimental phases. Mean CS valence ratings of study day 2 (graphs on the right) are again distinguished between placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions. Rating trial numbers indicate the exact trials of the experimental phases (Hab, habituation; Acq, acquisition training, Ext, extinction training; Rec, recall test; Reinst, reinstatement test) where CS valence ratings were provided. Dashed lines indicated the change from one experimental phase to the other during study day 1. Dotted lines mark the reinstatement manipulation phase at which the unconditioned stimulus (US) was applied without pairing to the CS+ on study day 2 in order to investigate extinction efficacy or possible reinstatement effects. Please note that negative values indicate positive CS valence ratings (i.e., pleasantness), while positive values represent negative CS valence (i.e., unpleasantness). CS, conditioned stimulus.

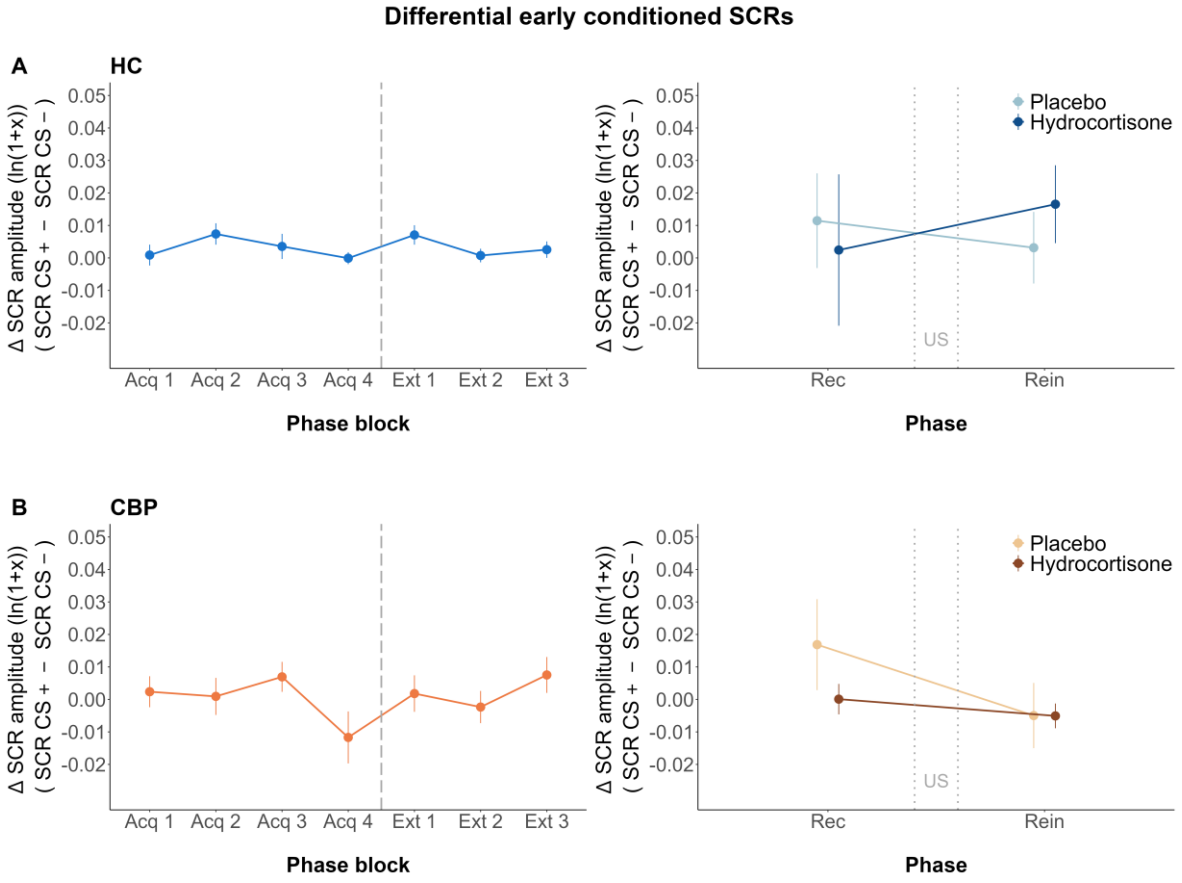

Figure 4. Differential early conditioned skin conductance responses (first interval responses, FIR). Mean differential (Δ) early conditioned skin conductance responses (SCRs), (SCR amplitudes CS+ - SCR amplitudes CS-) ± standard error of the mean (SEM) of patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue) throughout all experimental phases. On study day 2, mean differential early conditioned SCRs are divided into placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions. Please note, that due to the different analysis strategies on study day 1 and 2, the visualization of the ΔSCRs differs a bit between both study days. On study day 1, the main interest focused on the acquisition and extinction learning dynamics of the two experimental groups (CBP, HCs). Therefore, each phase block includes 3 ΔSCRs resulting in four different phase blocks for the acquisition training (Acq 1-4) and three phase blocks for the extinction training (Ext 1-3). On study day 2, the focus was set on the extinction efficacy/reinstatement effect and its modulation by pharmacologically induced stress via hydrocortisone treatment. Therefore, SCRs of the recall test (Rec) were directly compared to the SCRs of the reinstatement test phase (Rein). The dashed line separates different experimental phases of study day 1, while the dotted lines indicate the reinstatement manipulation phase on study day 2 (unannounced application of the unconditioned stimulus (US) in order to induce reinstatement effects). CS, conditioned stimulus.

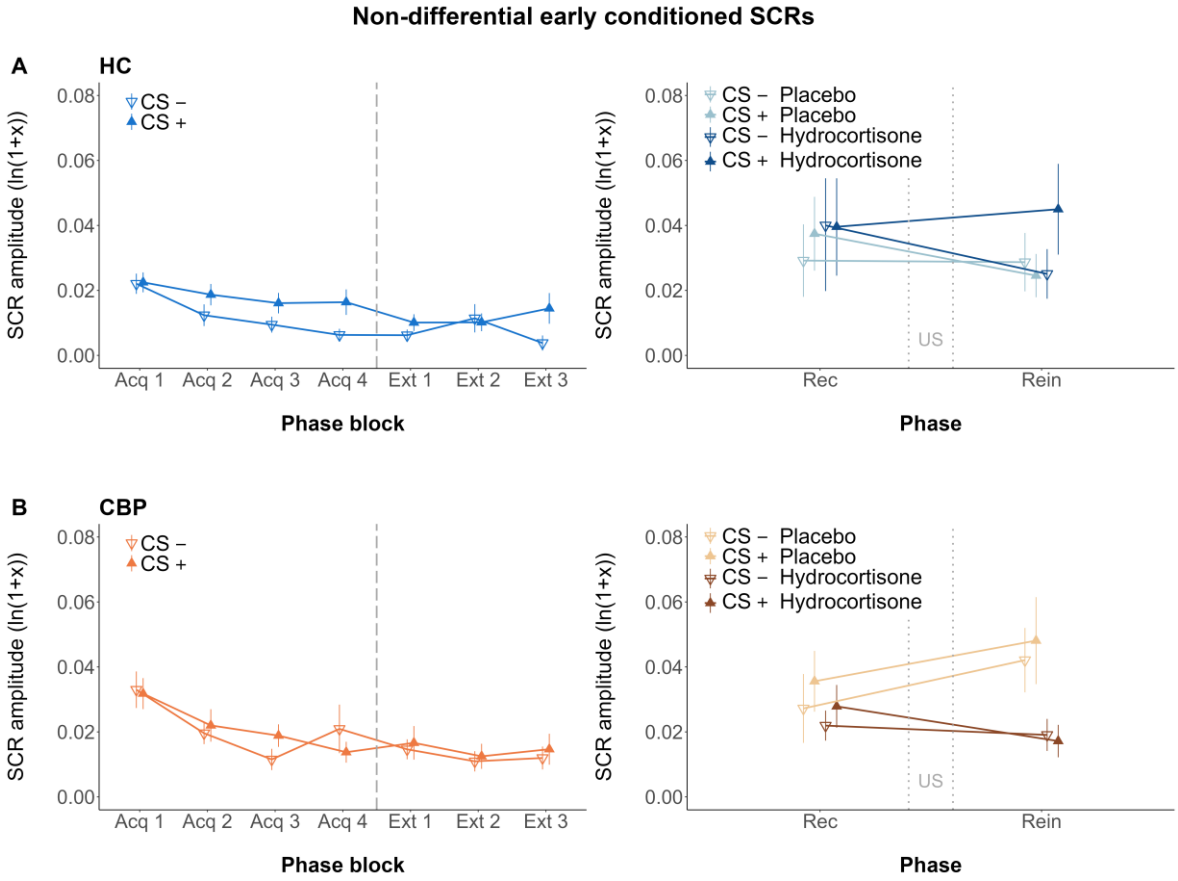

Figure 5. Non-differential early conditioned skin conductance responses (first interval responses, FIR). Mean early conditioned skin conductance response amplitudes (SCRs) ± standard error of the mean (SEM) are depicted for patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue) including their reactions to the CS+ (filled triangles) and to the CS- (unfilled upside-down triangles) over the course of both study days. For study day 2, the participants' mean early conditioned SCRs are distinguished between placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions. As a reminder, due to the different analysis strategies that were applied for the SCR data of study day 1 and 2, SCRs are differently visualized for study 1 and 2. On study day 1, the main focus lay on the acquisition and extinction learning dynamics of both experimental groups (CBP, HCs). Therefore, each phase block includes up to 3 SCRs of each participant resulting in four different phase blocks for the acquisition (Acq 1-4) and three phase blocks for the extinction training (Ext 1-3) phase. The focus on study day 2 lay on the investigation of extinction efficacy/reinstatement effects and their modulation by pharmacologically induced stress (via hydrocortisone) in both experimental groups. Thus, SCRs of the recall test (Rec) were directly compared to the SCRs of the reinstatement test phase (Rein). The different experimental phases of study day 1 are separated by the dashed line. The dotted lines frame the reinstatement manipulation phase of study day 2, where the unconditioned stimulus (US) was presented without any CS presentation to induce reinstatement effects. CS, conditioned stimulus.

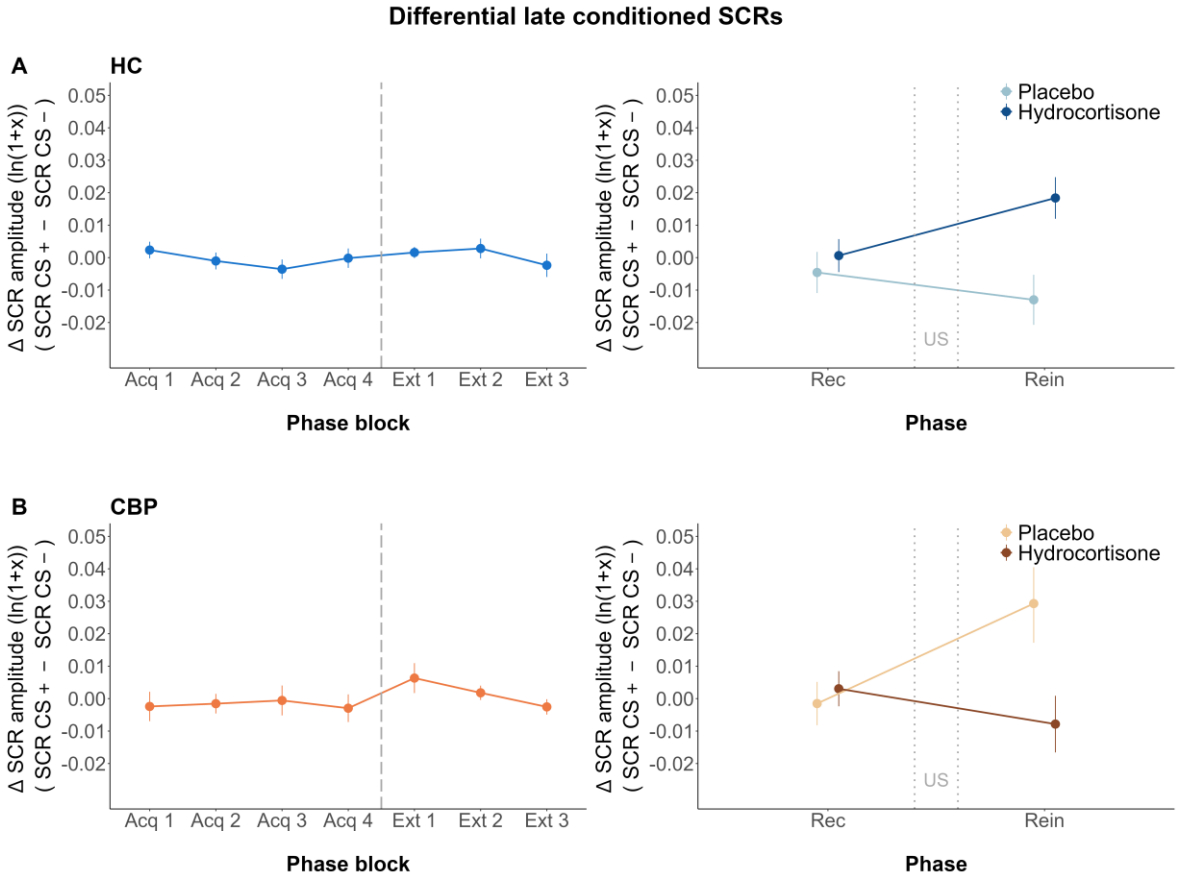

Figure 6. Differential late conditioned skin conductance responses (second interval responses, SIR). Mean differential (Δ) late conditioned skin conductance responses (SCRs), (SCR amplitudes to the CS+ - SCR amplitudes to the CS-) ± standard error of the mean (SEM) of patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue) are displayed for study day 1 and 2. Mean differential early conditioned SCRs of study day 2 are divided into placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions. Please remember, that due to different analysis strategies for the data of study day 1 and 2, ΔSCRs are depicted in a different manner on study 1 and 2. Since the main interest for the analysis of the study day 1 data was focused on the learning dynamic during the acquisition and extinction training phases, each phase block consists of 3 ΔSCRs resulting in four different phase blocks for the acquisition (Acq 1-4) and three phase blocks for the extinction training (Ext 1-3). On study day 2, the focus lay on the investigation of the extinction efficacy/reinstatement effect and its modulation by pharmacologically induced stress via hydrocortisone treatment in both experimental groups (CBP, HCs). Thus, SCRs of the recall test (Rec) were directly compared to the SCRs of the reinstatement test phase (Rein). For the study day 1 data, different experimental phases are separated by the dashed line. The dotted line marks the reinstatement manipulation phase of study day 2, where participants received an unannounced application of the unconditioned stimulus (US) in order to induce reinstatement effects. CS, conditioned stimulus.

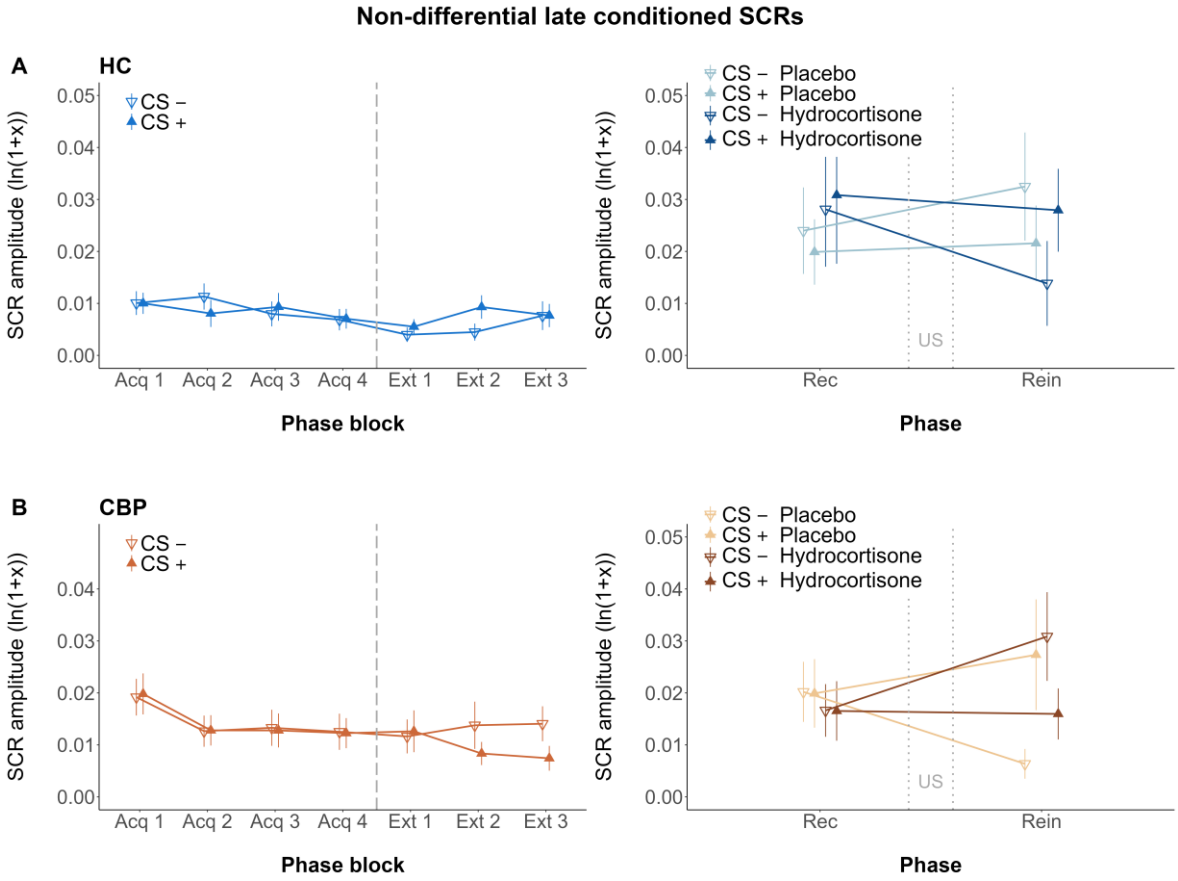

Figure 7. Non-differential late conditioned skin conductance responses (second interval responses, SIR). Mean late conditioned skin conductance response amplitudes (SCRs) ± standard error of the mean (SEM) are shown for patients with non-specific chronic back pain (CBP, orange) and pain-free healthy control participants (HCs, blue). Their reactions to the CS+ (filled triangles) and to the CS- (unfilled, upside-down triangles) are depicted for both study days. For study day 2, mean late conditioned SCRs are separated in placebo (CBP, light orange; HCs, light blue) and hydrocortisone (CBP, brown; HCs, dark blue) treatment conditions. Please note that due to the different analysis strategies applied for the SCR data of the different study days, SCRs are differently visualized for study 1 and 2. The main interest for the study day 1 data was focused on the acquisition and extinction learning dynamics of both patients with CBP and HCs. Hence, each phase block includes up to 3 SCRs of each participant resulting in four different phase blocks for the acquisition (Acq 1-4) and three phase blocks for the extinction training (Ext 1-3) phase. The main interest for the study day 2 data lay on extinction efficacy/reinstatement effects in both experimental groups and their modulation by pharmacologically induced stress (via hydrocortisone). Therefore, SCRs of the recall test (Rec) were directly compared to the SCRs of the reinstatement test phase (Rein). The dashed line separates the different experimental phases of study day 1, while the dotted lines delineate the reinstatement manipulation phase of study day 2, where the unconditioned stimulus (US) was applied without any CS presentation to induce reinstatement effects. CS, conditioned stimulus.

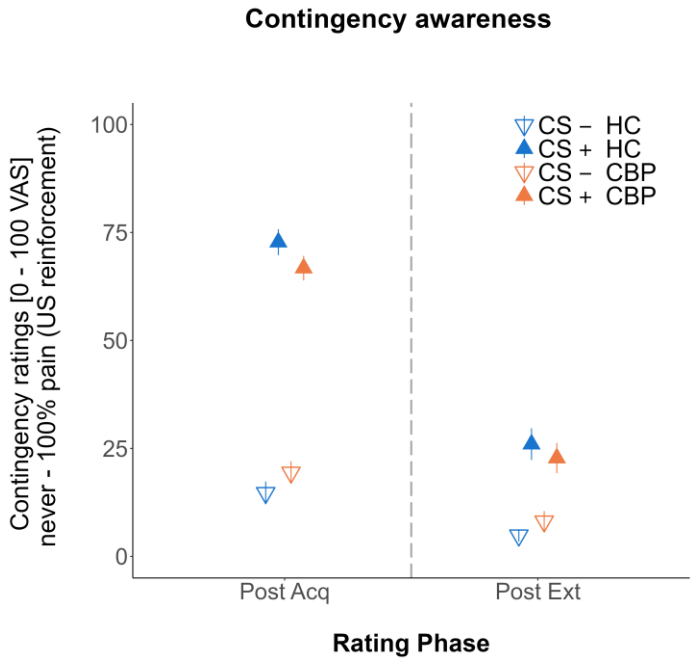

Figure 8. Contingency awareness. Mean contingency ratings ± standard error of the mean (SEM) that were provided by the patients with non-specific chronic back pain (CBP, orange) and the pain-free healthy control participants (HCs, blue) with regard to the pairing with the US for the CS+ (filled triangles) and the CS- (unfilled, upside-down triangles) after the acquisition (Post Acq) and extinction training (Post Ext), respectively. The dashed line separates the two experimental phases. CS, conditioned stimulus.

3.5. Cortisol Levels

Cortisol levels were analyzed in order to control for the effect of hydrocortisone on previously developed memory traces in terms of pain-related acquisition and extinction learning, and the reinstatement of the pain-related acquisition memory.

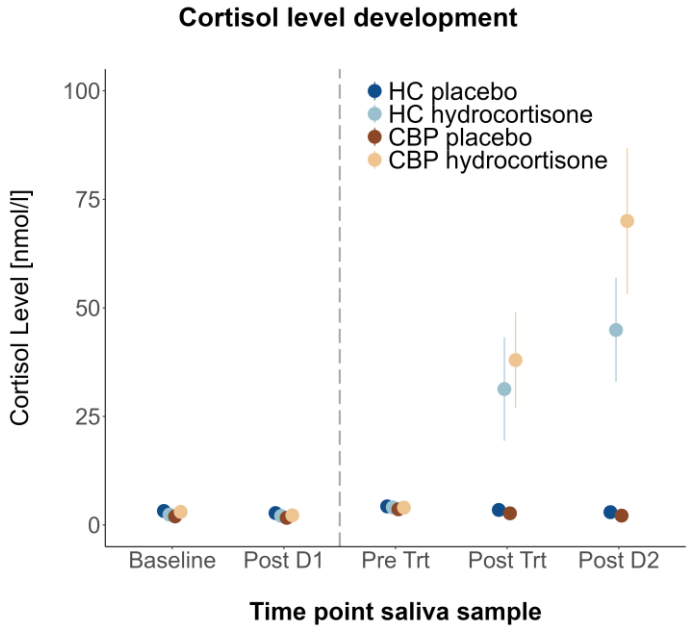

Figure 9. Cortisol level development. Mean cortisol levels in nanomol (10^{-19} mol) per liter (nmol/l) ± standard error of the mean (SEM) of patients with non-specific chronic back pain (CBP, brown) and pain-free healthy control participants (HCs, blue) of the placebo (CBP, tan; HCs, turquoise) throughout both study days. On study day 1 (D1), saliva samples were collected before (Baseline) and after (Post D1) the experiment. On study day 2 (D2), participants supplied their saliva samples right before the treatment (Pre Trt), ~45 minutes after the treatment/just before the experiment (Post Trt) and just after the experiment (Post D2). The both study days are separated by the dashed line.

Cortisol levels differentially changed from pre-treatment to post-experiment in the hydrocortisone treatment groups of both experimental groups (patients with CBP, HCs) as compared to the placebo groups (IA *time* × *treatment*: patients with CBP: $F(1,57) = 14.61$, $p < 0.001$, $\eta_p^2 = 0.20$; HCs: $F(1,55) = 10.61$, $p = 0.002$, $\eta_p^2 = 0.16$) as depicted in *Figure 9 – Cortisol level development*. In detail, cortisol levels significantly increased in in the hydrocortisone treatment groups, while placebo treatment groups revealed a significant

decrease in their cortisol levels (patients with CBP_{hydro}: $t(30) = 3.94$, $p < 0.001$, $d = 1.44$; HC_{hydro}: $t(30) = 3.45$, $p < 0.001$, $d = 1.26$; patients with $CBP_{placebo}$: $t(27) = -5.43$, $p < 0.001$, $d = -2.09$; $HC_{placebo}$: $t(25) = -5.43$, $p < 0.001$, $d = -2.17$). No significant differences between experimental groups were found in neither treatment condition (all $p > 0.05$).

3.6. Recall Test

Valence ratings. Comparing ΔCS valence ratings of the extinction training with ΔCS valence ratings of the recall test phase (see *Figure 2. Differential CS valence ratings*), neither significant MEs nor IAs of the factors *group, treatment,* and *time* were found (all $p > 0.05$). On the other hand, non-differential analyses of the CS valence ratings (*Figure 3. Non-differential CS valence ratings*) revealed a *time* × *group* IA on a trend level ($F(1,221.12) = 3.21$, $p = 0.07$, η_p^2=0.01) which further uncovered that HCs CS valences of both CS types (i.e., CS+, CS-) showed a stronger negative shift compared with CS valences of patients with CBP across both treatment groups (ME *time*: patients with CBP: $F(1,109.81) = 6.66$, $p = 0.01$, $\eta_p^2 = 0.06$; HCs: $F(1,111.28) = 18.76$, $p < 0.001$, $\eta_p^2 = 0.14$). In addition, valence ratings of both CS types differed significantly in both the extinction training and recall test phase as indicated by a ME for *CS type* ($F(1,119.03) = 12.75$, $p < 0.001$, $\eta_p^2 = 0.10$). Thereby, this ME was found to be dependent on valence ratings of the HCs only, while patients with CBP did not reveal any differences in valence ratings of the different CS types (HCs: $F(1,60.60) = 12.87$, $p < 0.001$, $\eta_p^2 = 0.18$; patients with CBP: $F(1,57.90) = 2.17$, $p = 0.15$, $\eta_p^2 = 0.04$). No further MEs or IAs were observed.

Skin conductance responses. According to ΔCS valence ratings, no significant MEs or IAs were found in ΔSCRs (*Figure 6. Differential late conditioned skin conductance responses (second interval responses, SIR)*) for the factors *time, group,* and *treatment* (all $p > 0.05$). Non-differential analyses of SCRs, however, uncovered a ME for the factor *time* showing increasing SCRs for both CS types in both treatment groups of the HCs only (*Figure 7. Non-differential late conditioned skin conductance responses (second interval responses, SIR)*), comparing SCRs at the end of the extinction training to SCRs at the beginning of the recall

test phase (F(1,48.06) = 8.02, p = 0.007, η_p^2 = 0.14). Analyses of the SCRs of patients with CBP did not reveal any MEs or IAs (all $p > 0.05$).

3.7. Reinstatement Manipulation

Neither mean pain intensity ratings nor US-related SCRs differed between groups or treatment conditions (no significant MEs or IAs of the factors *group* and *treatment*, all $p > 0.05$) during the reinstatement manipulation phase. The latter significantly decreased over the course of the reinstatement manipulation in both experimental and treatment groups as indicated by a ME *time* (F(1,108.27) = 51.62, $p < 0.001$, η_p^2 = 0.34).

3.8. Reinstatement Test

Valence ratings. The analysis of ΔCS valence ratings did not reveal any significant MEs or IAs of the factors *group*, *time*, and *treatment* (*Figure 2. Differential CS valence ratings*). As depicted in *Figure 3. Non-differential CS valence ratings*, non-differential analyses yielded different changes of CS+ and CS- valence ratings from the end of the recall test to the beginning of the reinstatement test phase in both HCs treatment groups on a trend level. Patient valence ratings on the CS+ and CS-, on the other hand, changed in opposite directions (IA *time* × *CS type* × *group* × *treatment*: F(1,217.74) = 2.94, p = 0.09, η_p^2 = 0.01; patients with CBP: IA *time* × *CS type*: F(1,107.73) = 2.92, p = 0.09, η_p^2 = 0.03; HCs: IA *time* × *CS type* × *treatment*: F(1,110.12) = 3.15, p = 0.08, η_p^2 = 0.03).

Skin conductance responses. The placebo and hydrocortisone treatment groups of both patients with CBP and HCs showed changes in their ΔSCRs that significantly differed between both treatment groups (*Figure 6. Differential late conditioned skin conductance responses (second interval responses, SIR)*). As already indicated in the non-differential analyses of CS valence ratings, this effect occurred in opposite directions in both patients with CBP and HCs (IA *time* × *group* × *treatment*: F(1,118) = 10.94, p = 0.001, η_p^2 = 0.08; IA *time* × *treatment*: patients with CBP: F(1,57) = 6.75, p = 0.01, η_p^2 = 0.11; HCs: F(1,37.56)

= 4.37, p = 0.04, η_p^2 = 0.10). In detail, post hoc *t*-tests uncovered significantly increasing ΔSCRs comparing the recall to the reinstatement test phase in the placebo treatment group of patients with CBP ($t(30) = 2.49$, $p = 0.01$, $d = 0.91$). ΔSCRs of the HCs' placebo treatment group, did not change significantly. On the other hand, in the hydrocortisone treatment group of HCs, ΔSCRs were observed to significantly increase ($t(19.61) = 3.10$, $p = 0.002$, $d = 1.40$), while ΔSCRs of the patients' hydrocortisone treatment group did not change significantly.

Further, non-differential analyses revealed significantly different changes in SCRs to both CS types in both experimental and treatment groups (IA *time* × *CS type* × *group* × *treatment:* $F(1,158.09) = 12.01$, $p < 0.001$, $\eta_p^2 = 0.07$; IA *time* × *CS type* × *treatment*: patients with CBP: $F(1,108.67)=8.91$, $p=0.004$, $\eta_p^2=0.08$; HCs: $F(1,75.41) = 3.82$, $p = 0.05$, $\eta_p^2 = 0.05$). In detail, when comparing SCRs to the different CS types of the recall test with the reinstatement test phase, non-differential analyses uncovered a significant decrease of SCRs to the CS- in patients with CBP that were in the placebo treatment group (IA *time* × *CS type*: $F(1,45.32) = 6.49$, $p = 0.01$, $\eta_p^2 = 0.13$; post hoc *t*-test: $t(23.35) = -2.00$, $p = 0.045$, $d = -0.83$), while SCRs to the CS+ did not change. No changes were observed in the development of SCRs to neither CS type in patients with CBP that received the hydrocortisone treatment. In contrast, HCs that received the placebo treatment, did not show any changes in the development of their SCRs to the different CS types, while in HCs that were in the hydrocortisone treatment group SCRs to the CS- decreases significantly from the recall to the reinstatement test phase and SCRs to the CS+ did not change in this treatment group (IA *time* × *CS type*: $F(1,41.87) = 3.97$, $p = 0.05$, $\eta_p^2 = 0.09$; post hoc *t*-test: $t(23.32) = -1.94$, $p = 0.05$, $d = -0.81$) as visualized in *Figure 7 – Non-differential late conditioned skin conductance responses (second interval responses, SIR)*. There were no further significant MEs or IAs in neither experimental nor treatment group (all $p < 0.05$).

Data analysis of the valence ratings and SCRs of study day 2 did not reveal any modulation effect of the tested covariates neither in HCs nor in patients with CBP (all $p > 0.05$).

4. Discussion

This thesis investigated potential alterations in the acquisition and extinction of pain-related threat and safety signals (study day 1) in n = 62 patients with non-specific chronic back pain (CBP) and n = 61 age- and gender-matched pain-free healthy control participants (HCs). Further, the modulating role of pharmacologically induced stress on the retrieval of extinction memory (i.e., extinction efficacy) and the reinstatement of the former acquired pain-related threat and safety associations (i.e., relapse) was tested and compared within and between both experimental groups (study day 2). Therefore, a 2-day classical differential conditioning paradigm with geometric figures as conditioned stimuli (CS) that predicted the occurrence (CS+) or omission (CS-) of a phasic heat pain stimulus (i.e., the unconditioned stimulus, US) was used. On the second study day, participants received 20mg of hydrocortisone in a double-blind, pseudo-randomized, placebo-controlled fashion before testing recall and reinstatement effects. Main outcomes were individual CS valence ratings as a self-reported behavioral, evaluative measure for implicit emotional learning, CS-US contingency ratings as a measure for explicit learning, and skin conductance responses (SCRs) as a physiological measure.

Overall, the results reported in this thesis can confirm and extend recent findings of this research field. (i) First, patients with CBP revealed impaired differential pain-related learning as compared with HCs during acquisition training. Here, both threat (CS+) and safety (CS-) learning were affected. (ii) Over the course of the extinction training, pain-related threat associations decreased in both groups, while there was no extinction to the safety signal in neither group. (iii) Patients that received placebo treatment revealed a differential reinstatement of formerly acquired pain-related associations, while patients that received hydrocortisone did not. (iv) In contrast, HCs that were treated with hydrocortisone exhibited a differential reinstatement, while HCs that received placebo did not. In both groups, reinstatement effects were driven by their reactions to the safety signal. (v) The presented data further provide evidence for a modulating role of different pain-related variables such as pain duration, pain-related anxiety, and pain catastrophizing in patients with CBP, and further, state anxiety (both patients and HCs) and arousal (only HCs) on pain-related learning processes. In the following sections, the aforementioned results will be thoroughly discussed.

4.1. Successful acquisition and extinction learning about the pain-related predictive functions of threat and safety signals in patients with non-specific CBP and HCs.

In general, the reported observations confirm previous reports about successful pain-related threat and safety learning in pain-free healthy participants and patients suffering from different chronic pain conditions (Harvie et al., 2020; Icenhour et al., 2015; Meulders et al., 2018, 2017). While in both experimental groups valences of the threat signals became more unpleasant, valences of the safety signal became more pleasant during acquisition training. Thus, HCs and patients with non-specific CBP apparently developed emotional associations towards the visual cues and learned about their US-predictive function. This was additionally confirmed by the CS-US contingency ratings collected at the end of the acquisition training.

Further, both groups demonstrated successful extinction learning of their threat signal associations by providing more pleasant valence ratings during extinction training that even returned to the values of the habituation phase. Valence ratings towards the safety cue did not change in neither group. This has been reported earlier in studies investigating healthy participants using valences ratings as an evaluative measure (Blechert et al., 2007; Rothemund et al., 2012). Considering the fact that the former safety cue did not gain a new, e.g., threatening, function, it seems reasonable that safety cue valences did not return to their former values during extinction training. Predictive measures such as expectancy ratings instead of an emotional evaluative measure (i.e., valence ratings) might have better captured extinction learning in this experimental design, especially for the safety cue (Haaker et al., 2014). However, contingency ratings after extinction training confirmed the successful re-learning of the new CS-US contingencies in both groups.

Thus, the intrinsic associative processes that are needed for implicit emotional and explicit learning (captured by valence and contingency ratings, respectively), and further, re-learning (i.e., extinction learning) generally seem to work in both HCs and patients with non-specific CBP. The mechanisms of extinction learning, which is discussed by Hartley and Phelps (2012) to be the formation of new memory traces that inhibit rather than replace former acquired and established memory traces, are primarily applied in exposure therapies that are increasingly implemented in the treatment of chronic pain (Gatzounis et al., 2021). Hence,

an inability to develop novel safety associations that inhibit former threat associations would be fatal for patients and diminish their hope for successful treatment.

4.2. The influence of hydrocortisone on the retrieval of extinction memory and the reinstatement of former acquired pain-related threat and safety associations in HCs.

To further investigate the influence of pharmacologically induced stress/hydrocortisone on the retrieval of extinction memory and the reinstatement of previously acquired pain-related threat and safety associations, and thus on extinction efficacy and relapse phenomena in HCs, and to later compare them with the patients, it was first essential that both groups respond equally to its administration (manipulation check). Therefore, saliva samples were collected on both study days to track the participants' cortisol levels. Importantly, neither baseline values on study day 1 nor study day 2 differed between later treatment groups or between HCs and patients. As expected, cortisol levels of participants who received hydrocortisone increased significantly and comparable to other studies (Hagedorn et al., 2021; Merz et al., 2013). In contrast, cortisol levels of participants who received placebo slightly decreased.

In this study, neither a clear retrieval of extinction memory nor spontaneous recovery of former acquired pain-related associations could be observed in the recall test. Valence ratings of both the threat and safety cue significantly increased in each group. In HCs, this was further supported by their SCRs. This finding could indicate a general alertness/arousal of both, patients and HCs of both treatment conditions, who might still remembered the US of the first study day and have expected it to return during the recall test, without knowing whether it now would be paired with the former threat or safety cue.

Further on, after the unannounced presentation of the previous painful heat stimulus during the reinstatement manipulation, a reinstatement effect could be observed for hydrocortisone-treated HCs during the reinstatement test as indicated by statistically significant increases in their differential SCRs and further supported by their valence ratings on a trend level. It was mainly driven by decreasing SCRs to the safety signal while the SCRs to the threat signal did not change, which suggests that the CRs to the safety signal diminished, while CRs to the

threat signal remained stable. Hydrocortisone thus might have triggered the reinstatement of the initially formed memory trace about the US-predictive functions of the visual cues after a spontaneous, unexpected encounter with the US, thereby attenuating the later acquired extinction memory trace. This finding further supports previous reports of stress-induced effects on extinction memory retrieval and the reinstatement of former acquired conditioned fear responses in healthy participants (Raio et al., 2014) as postulated in the earlier introduced *STaR model* of Meir Drexler et al. (2019a) (see *1.4.2. Stress in human extinction learning studies and the role of its timing*). There, stress, applied prior to a recall test, is discussed to disrupt extinction memory retrieval (i.e., extinction efficacy) and to promote reinstatement of previously formed pain-related acquisition/fear memory (i.e., relapse), which can now be extended by the modality of pain and patients with non-specific CBP.

Contrary to the previously stated expectations, no reinstatement effect was observed in placebo-treated HCs. Indeed, other studies that examined non-treated healthy participants using this type of classical differential conditioning paradigms actually observed only small or even no reinstatement effects in valence ratings (Schmidt et al., 2020) or SCRs (Icenhour et al., 2015) either. Moreover, the induction of reinstatement effects in the context of fear conditioning is not straightforward in general and does not always succeed (Haaker et al., 2014), but it seems that this effect becomes more apparent when using hydrocortisone and exposing (healthy) participants to (pharmacologically induced) stress.

In the end, it remains open why SCRs, rather than valence ratings, were the driving force in detecting stress-/hydrocortisone-induced reinstatement effects in this study. One possible reason might be that complex reinstatement effects are more easily reflected in physiological responses to the CS than evaluative, self-report measures like CS valence ratings. Future studies could thus consider additional measures, e.g., US-expectancy ratings or pupillometry measures in order to better detect reinstatement effects.

4.3. Alterations in patients with non-specific chronic back pain and their consequences – more proneness to relapse?

This section thoroughly discusses the findings of altered differential emotional threat and safety learning, as well as reinstatement phenomena in patients with non-specific CBP.

Despite the fact that the patients were in principle able to recognize and learn the predictive functions of the threat and safety cues within the classical differential conditioning paradigm used here, their differential emotional learning (related to valence ratings) was significantly attenuated as compared to the HCs. This confirms evidence of impaired differential learning in patients with different chronic pain disorders (Harvie et al., 2017).

Impaired safety learning has previously been reported for patients with chronic hand (Meulders et al., 2014), and neck pain (Harvie et al., 2020). However, our data indicates deficits in emotional threat and safety learning in patients with non-specific CBP. Until now, only Harvie et al. (2020) reported about such a global impairment of threat and safety learning in patients with chronic neck pain. Contrary to previous studies (Jentsch et al., 2020; Merz et al., 2016), the SCRs neither supported nor contradicted the behavioral findings of the acquisition training here.

For the complex process of chronification and the maintenance of the chronic pain state, not only alterations in the acquisition, but also in the extinction of pain-related emotions could be of relevance. In the present study, valence ratings on the threat and safety cue were expected to converge to former baseline values during extinction training, where no US was applied. In fact, the change of the threat cue valences becoming more pleasant again, occurred to be slower in patients as compared to HCs. Such a general deceleration in extinction learning may be a first indicator of impaired extinction learning processes in patients with CBP, and, furthermore, may have a negative impact on extinction efficacy, which could in turn lead to an eased relapse or reinstatement of former pain-related CRs. Hence, the so far only scarce knowledge related to possibly altered extinction learning processes in chronic pain disorders can now be extended. While patients with irritable bowel syndrome showed comparable extinction learning to HCs (Icenhour et al., 2015), patients with fibromyalgia

revealed impaired extinction learning on generalized stimuli (Meulders et al., 2017). However, it could also be possible that this finding is driven by a 'floor effect' due to the patients' weaker threat learning during the acquisition training (i.e., flatter slopes). Thus, they might not require as strong extinction learning as the HCs to return to former baseline values. In order to draw more meaningful conclusions regarding possible extinction learning deficits of patients with non-specific CBP, threat and safety learning would have been needed to be similarly strong in both groups after acquisition training.

Interestingly, a differential reinstatement of pain-related associations was observed in placebo-treated patients, but not in placebo-treated HCs. This effect, however, was statistically significant only for the patients' differential physiological responses (i.e., SCRs) and was supported descriptively by their valence ratings. As for the hydrocortisone-treated HCs before, this effect was driven by a significant decrease of the patients' SCRs to the safety signal, while their SCRs to the threat signal did not change. This may suggest that placebo-treated patients did not expect a painful heat stimulus for the safety signal and were thus less aroused during its presentation, whereas for the threat signal they still remembered the possibility of a painful heat stimulus and in turn may have been more aroused during its presentation. In contrast, hydrocortisone-treated patients did not reveal a reinstatement effect neither in their SCRs nor in their valence ratings. Patients in their natural state (i.e., placebo group) thus may be more prone to relapse phenomena than pain-free healthy individuals, possibly due to attenuated capacities to form a robust extinction memory. In order to translate these findings into therapeutic and thus, beneficial approaches, future studies should catch up on the underlying (neural) mechanisms of these impairments. Taken together, deficits to adequately distinguish novel safety and threat signals, and further, a higher risk of relapse due to attenuated extinction processes or extinction efficacy are a critical but commonly reported issue for patients with chronic pain (Turk and Rudy, 1991).

Especially the deficit to adequately evaluate a (novel) safety cue in a constant environment could possibly indicate some kind of overgeneralization like in other chronic pain pathologies (Harvie et al., 2020; Meulders et al., 2014) and contribute to the development and maintenance of chronic pain. Together with attenuated extinction learning processes, this

could further promote exaggerated and recurring maladaptive behaviors, such as a preference for movements or situations that patients know are safe and will not cause pain, resulting in relieving postures, disuse, and avoidance behavior. As a consequence, patients are often not able to leave the former described vicious circle of chronic pain (see *1.2. Learning mechanisms in the context of pain*), that was originally introduced by Vlaeyen and Linton (2000), without professional help. This might explain why it remains a long and difficult process for the patients to successfully respond to, e.g., exposure therapies. However, generalization processes or avoidance behavior were not examined here, but future studies are encouraged to address these processes, as their alterations could be highly relevant for the development and maintenance of chronic pain.

Impaired differential learning of pain-related emotions could be further associated with altered emotional and cognitive processing. Together with potential changes in an individual's personality (Gustin et al., 2014), this could, e.g., rely on structural changes in brain networks related to emotion regulation and cognitive functioning such as decision making, learning, and memory (Cauda et al., 2014; Kang et al., 2019; Shi et al., 2016), but also on altered brain activity (Baliki et al., 2006; Hashmi et al., 2013) and neurochemical changes, e.g., altered homeostasis of the dopaminergic system (Serafini et al., 2020) as reported in several studies examining patients with chronic pain. This, however, was not targeted here, but should be considered in future studies as well.

In this study, further evidence is provided that the length of pain duration is associated with impairments in both emotional (CS valence) and cognitive (contingency awareness) threat learning (Schlitt et al., 2021). This might indicate that these changes are steadily increasing over time and contribute to the maintenance of chronic pain, but whether reduced threat learning occurs within the chronification process or was already present before has to be investigated, e.g., in longitudinal studies. The fact that modulations of pain duration only addressed patients' threat learning might indicate a maladaptive mechanism in the patients, mainly directing their attention to cues signaling potential threat as discussed for the reinstatement findings before. In fact, according to Crombez et al. (2015), many studies could demonstrate an attentional bias towards pain-related information in patients with chronic

pain, but the findings also do not always point into one clear direction. All together, these results demonstrate the complexity of this disease. Many other factors like pain duration or maladaptive pain-related cognitions (i.e., pain anxiety, pain catastrophizing) and other psychological factors (e.g., state anxiety), were further found to differently influence the HCs' and the patients' pain-related learning, respectively. The patients' treatment thus should be based on their individual pain-related characteristics and their psychological state.

Another open point is the inverse influence of hydrocortisone on the reinstatement of pain-related associations in patients with non-specific CBP and HCs. Contrary to the HCs, there was no observable reinstatement of former acquired pain-related associations in patients that received hydrocortisone. This could be discussed in the light of possible alterations in the glucocorticoid system and in glucocorticoid sensitivity of patients with chronic pain (Geiss et al., 2012; Nees et al., 2019). Supporting this, patients in this sample exhibited increased chronic subjective stress levels compared to HCs (see *Table 3. Pain-related, fear, arousal, and self-report measures*). As stated in the introductory section of this thesis (see *1.4.1. The relationship between stress, chronic pain, and learning behavior*), an interaction between stress and pain is increasingly thought to catalyze pain chronification (Timmers et al., 2019). Here, hydrocortisone mainly affected the patients' CRs to the safety signal. Changes in the safety signal perception and thus, in the ability to adequately distinguish threat from safety, may impact the therapeutic treatment success and potentially support the maintenance of chronic pain (Vlaeyen, 2015). Therefore, therapeutic treatment should include the assessment of the patient's current and chronic stress levels as well.

All this could be applied, e.g., in the context of an interdisciplinary multimodal pain therapy (IMST), which is an evidence-based therapy approach and the so-called gold standard in chronic pain treatment (Kaiser et al., 2015; Pfingsten et al., 2019). Here, as outlined in Pfingsten et al. (2019), various therapeutic procedures (somatic, physical, and psychological) are individually tailored to the patient in a predefined treatment plan according to a therapeutic goal that all involved therapists have agreed on.

4.4. Limitations

The findings obtained in this study have to be interpreted in the light of several limitations. (I) The assessment of repeated CS valence ratings during the experimental phases (in order to track the temporal learning dynamics) might increase the awareness of the CS-US pairing and may thus interfere with the natural course of these learning dynamics, which is usually more subconscious in its nature (Lonsdorf et al., 2017). (II) By using short, moderately painful US, we successfully observed changes in self-reported valence and contingency ratings, and further in the physiological responses of both groups. However, within this rather artificial setting the conditions pain vs. no pain are easy to distinguish, while in the case of clinical pain with its natural fluctuations, learning to predict which cues or situations signal pain exacerbation or (at least partial) relief might be more challenging for the patients. (III) In addition, the relatively short two day classical differential conditioning paradigm used here can only provide a short-term snapshot rather than lead to long-term conclusions, which would be of particular relevance for patients suffering from non-specific CBP due to the overall long pain duration that most patients endure. Longitudinal studies are therefore required to draw more valid conclusions. (IV) Further, although the painful heat stimuli were equally painful in both groups, the ecological validity and relevance of the heat pain stimulus might have differed between the pain-free HCs and the patients. Chronic (back) pain is more characterized by fluctuations and less predictability than more easily predictable heat pain stimuli of consistent intensity to the volar forearm. (V) The patients that were investigated in this study were generally less affected (e.g., regarding pain duration, disability, and pain medication) as compared to patient samples of other studies (Harvie et al., 2020; Klinger et al., 2010; Meulders et al., 2017, 2014). The findings of this study might therefore rather underestimate effects in more severely affected clinical populations. (VI) Finally, perceived stress, e.g., via acute stress ratings could have supported the reported observations as it could have been a complementary measure to the neuroendocrine measure of the participants' cortisol levels. However, it was not assessed in order to avoid the participants' focus on potential medication-induced (side/nocebo) effects.

4.5. Outlook for future studies

Clinical implications that were discussed in this section need to be interpreted in the light of the fact that these classical differential conditioning experiments are conducted in highly standardized and artificial laboratory settings. Hence, they are more hypothetical and need to be confirmed by studies in more clinical contexts. First studies, however, already test virtual reality approaches in order to make those highly standardized and artificial laboratory settings more realistic and to increase their ecological validity. Future studies could further try to use CS that are more meaningful to patients suffering from chronic pain and more comparable to their actual pain condition, e.g., certain fear-inducing movements or painful stimulations that are closer to their actual pain location, and compare different levels of pain (low, moderate, and high). In a new classical differential conditioning paradigm that was later established in healthy participants in the research group, a capsaicin cream is used to apply moderate, consistent painful heat stimuli so that pain exacerbation and relief can be investigated (van der Schaaf et al., 2022). This model might therefore be closer to the patient's chronic pain situation and is currently used for investigating pain exacerbation and relief learning mechanisms in patients suffering from non-specific CBP (preregistered at the German Clinical Trial Register; ID: DRKS00027448). Finally, long-term studies could be conducted comparing patients with acute, postoperative and chronic pain to target the question whether the impairments in pain-related learning behavior and the observed increased relapse potential are already present before or whether they develop in the course of the chronification process. In this process anatomical and functional, e.g., resting-state, task-based functional magnetic resonance imaging (fMRI), or diffusion tensor imaging (DTI) data could be acquired, too, in order to investigate and possibly track changes that develop over time within these patient groups and to unravel the neural underpinnings contributing to the development and maintenance of chronic pain as well. Finally, novel machine learning approaches to predict individual pain-sensitivity based on individual resting-state brain connectivity (Spisak et al., 2020) could be further evolved to predict a general individual chronification risk to provide preventive therapy programs and to reduce the high individual and economic burden that is associated with chronic pain.

5. Conclusion

Overall, this work highlights the relevance of pain-related learning processes for the treatment of patients suffering from non-specific CBP and further, its impact on the patients' well-being and daily life. Impairments in differential pain-related threat and, especially, safety learning, combined with an increased relapse tendency could significantly contribute to the maintenance of the chronic pain state and further hamper treatment approaches as these are often based on extinction learning mechanisms. Limitations in the emotional processing of cues signaling threat or safety therefore may lead to excessive and maladaptive avoidance behavior, which hampers the patients to escape the vicious circle of pain-related anxiety, pain catastrophizing, avoidance, and disability without professional help. It does not mean that psychological treatment approaches, such as exposure therapies, are ineffective or meaningless. Rather, this shows that well-tailored treatment approaches, such as the interdisciplinary multimodal pain therapy, are needed in addition to standard therapy approaches in order to help patients develop a more robust extinction memory and increase extinction efficacy mechanisms to decrease or minimize the risk of relapse. Further findings of the patients' threat learning being modulated by the length of pain duration and the patients' degree of pain-related anxiety and pain catastrophizing strongly support the argument for individually tailored psychological therapy approaches based on disease specific and the patients' psychological characteristics. In addition, the observation about the different effects of the hydrocortisone treatment and therefore, pharmacologically induced stress, on the reinstatement of former acquired pain-related conditioned responses in pain-free healthy participants and patients with non-specific CBP underlines the complexity of this disease and supports the assumption that it has different psychological, pathophysiological, and neural underpinnings. Therefore, future studies are needed that further investigate those mechanisms together to unravel their complex relationship and further, their contribution to the development and maintenance of the non-specific CBP disease in order to improve existing treatment approaches or even generate entirely novel treatment strategies for the patients.

Summary

Non-specific chronic back pain (CBP) constitutes a tremendous social and psychological impact for patients, but also a major economic burden. In chronic pain, the biological warning function of acute pain is often missing. Although, altered pain-related learning mechanisms are thought to contribute to the development and maintenance of chronic pain, experimental research is scarce. For recovery, it is important to "unlearn" previously acquired pain-related associations through extinction learning processes and to develop robust extinction memory. Extinction learning might further be influenced by stress. Thus, this thesis investigated pain-related acquisition and extinction learning on one study day, and the influence of pharmacologically induced stress via oral hydrocortisone administration in a randomized placebo-controlled, double-blind design on the retrieval of extinction memory and the reinstatement of pain-related associations one day later in N = 62 patients with CBP and N = 61 healthy, pain-free, age- and sex-matched volunteers (healthy controls, HCs). In a two-day classical differential conditioning paradigm, geometric figures served as conditioned stimuli (CS) that predicted the appearance (threat cue, CS+) or absence (safety cue, CS-) of an unconditioned heat pain stimulus (US). Self-reported valence ratings were assessed as a behavioral measure for implicit evaluative learning, while contingency ratings covered its explicit aspect. Skin conductance responses (SCRs) were recorded as a physiological measure. Valence ratings of study day 1 indicate significantly impaired pain-related threat and safety learning in patients with CBP. SCRs of study day 2 showed hydrocortisone-induced reinstatement of pain-related associations in HCs, but not in patients. In contrast, placebo-treated patients revealed reinstatement of pain-related associations in their SCRs, while placebo-treated HCs did not. Thus, patients seem to have an increased relapse potential, which, together with the impaired pain-related threat and safety learning, may significantly contributes to the maintenance of the chronic pain state and complicates its treatment. Enhanced treatment approaches are thus strongly needed to help patients develop more robust extinction memory and reduce the risk of relapse. The unequal effects of hydrocortisone emphasize the complexity of the non-specific CBP disease and the need for additional research to unravel its different psychological, pathophysiological, and neural underpinnings.

Zusammenfassung

Unspezifische chronische Rückenschmerzen (RS) sind eine große soziale und psychologische Belastung für Patienten sowie eine enorme wirtschaftliche Last. Trotz der vermuteten Beteiligung veränderter schmerzbezogener Lernmechanismen an der Entstehung und Aufrechterhaltung chronischer Schmerzen gibt es hierzu bisher nur wenige Untersuchungen. Das für die Genesung relevante „Verlernen“ erworbener schmerzbezogener Assoziationen durch Extinktionslernen kann durch Stress beeinflusst werden. Diese Arbeit untersuchte schmerzbezogenes Erwerbs- und Extinktionslernen (Studientag 1) sowie den Einfluss von pharmakologisch induziertem Stress durch eine randomisierte, Placebo-kontrollierte, doppelblinde Hydrocortison-Gabe auf den Abruf des Extinktionsgedächtnisses und das Reinstatement schmerzbezogener Assoziationen (Studientag 2) bei N = 62 Patienten mit unspezifischen chronischen RS und N = 61 gesunden, schmerzfreien Teilnehmern. In einem klassischen, differentiellen Konditionierungsparadigma kündigten geometrische Figuren als konditionierte Reize das Auftreten oder Ausbleiben unkonditionierter Hitzeschmerzreizen an. Evaluative Valenzratings wurden als Verhaltensmaß für implizites und Kontingenzratings für explizites Lernen sowie elektrodermale Aktivität (EDA) als physiologisches Maß erhoben. An Studientag 1 zeigten die Valenzratings ein vermindertes Bedrohungs- und Sicherheitslernen bei den Patienten. An Studientag 2 wies die EDA ein Hydrocortison-induziertes Reinstatement schmerzbezogener Assoziationen bei Gesunden, aber nicht bei Patienten auf. Patienten der Placebo-Gruppe zeigten jedoch ein Reinstatement schmerzbezogener Assoziationen in der EDA, die Gesunden hingegen nicht. Patienten mit chronischen RS scheinen demnach ein erhöhtes Rückfallpotenzial zu haben, was zusammen mit vermindertem schmerzbezogenen Bedrohungs- und Sicherheitslernen elementar zur Aufrechterhaltung chronischer Schmerzen beitragen und deren Behandlung erschweren kann. Daher sind verbesserte Behandlungsansätze für Patienten dringend erforderlich, um ein robusteres Extinktionsgedächtnis auszubilden und das Rückfallrisiko zu verringern. Die ungleiche Wirkung von Hydrocortison in den beiden Experimentalgruppen unterstreicht die Komplexität chronischer RS und den Bedarf weiterer Forschung zur Entschlüsselung ihrer verschiedenen psychologischen, pathophysiologischen und neuronalen Grundlagen.

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List of Abbreviations

Abbreviation	Meaning
η_p^2	Partial eta-square
ACE	angiotensin-converting-enzyme
Acq	Acquisition (training)
ADS-K	Center for Epidemiological Studies-Depression Scale
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
ASS	German “Acetylsalicylsäure”, English “Acetylsalicylic acid”
AT1	angiotensin-II-type-1
BC	Before Christ
BO	German “Berufsordnung”, English “Professional code“
C	Celsius
CBP	Chronic back pain
CHEPS	Contact Heat-Evoked Potential Stimulator
CNS	Central nervous system
CR	Conditioned reaction
CS	Conditioned stimulus
d	Cohen’s *d* (effect size)
D1	Study day 1
D2	Study day 2
DASS	Depression Anxiety Stress Scales
Dr. med.	Latin “Doctor medicinae”, English “Doctor of Medicine”
e.g.	Latin “exempli gratia”, English “for example”
et al.	Latin “et alia”, English “and others”
etc.	Latin “et cetera”, English “and the rest”
Ext	Extinction (training)
F	Ratio of two variances (ANOVA)

FEDA	German “Fragebogen zu erlebten Defiziten der Aufmerksamkeit“, English „Center for Epidemiological Studies-Depression Scale“
FIR	First-interval response
GC	Glucocorticoid
h	Hour
Hab	Habituation (phase)
HC	Healthy control
HPA	Hypothalamic-pituitary-adrenal
Hz	Hertz
i.e.	Latin “it est”, English “that is”
IA	Interaction
IASP	International Association for the Study of Pain
Inc.	Incorporated
ITI	Inter-trial interval
kHz	Kilohertz
LMM	Linear Mixed Model
ln	Natural logarithm
M	Mean
ME	Main Effect
mg	Milligramm
N	Sample size
nmol	Nanomol
NRS	Numeric Rating Scale
NSAID	Non-steroidal anti-inflammatory drug
p	*p*-Value
PASS	Pain Anxiety Symptom Scale
PCS	Pain Catastrophizing Scale
PNS	Peripheral nervous system
Prof.	Professor
PSQ	Perceived Stress Questionnaire

RBG	Red Green Blue
Rec	Recall (test)
Reinst	Reinstatement (test)
s	Second
SCR	Skin conductance response
SD	Standard Deviation
SECPT	Socially Evaluated Cold Pressure Test
SEM	Standard Error of the Mean
SIR	Second-interval response
STADI	State-Trait-Anxiety-Depression-Inventory
STaR	Stress Timing affects Relapse
TICS	Trier Inventory for Chronic Stress
Trt	Treatment
TSST	Trier Social Stress Test
US	Unconditioned stimulus
USA	United States of America
VAS	Visual Analogue Scale
WHO	World Health Organization
μS	Micro Siemens

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Curriculum Vitae

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