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Emotional learning and its impact on back pain chronicity

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Zusammenfassung

Chronische Rückenschmerzen sind ein weltweites Gesundheitsproblem. Patienten mit chronischen Rückenschmerzen leben häufig in einem Teufelskreis, der von Leid und Unfähigkeit geprägt ist. Neuroimaging Studien konnten bereits zeigen, dass mit dem chronischen Schmerz sowohl funktionelle, wie auch strukturelle Veränderungen des Gehirns einhergehen. Trotz dieser Erkenntnisse ist noch immer unklar, ob diese Veränderungen auch Prädiktoren für die Chronifizierung des Schmerzes sind und welche Mechanismen im Gehirn diesem Prozess zugrunde liegen. In diesem Kontext könnten emotionale Lernprozesse eine wichtige Rolle spielen. Die Untersuchung der den strukturellen und funktionellen Veränderungen im Gehirn zugrunde liegenden Mechanismen könnten helfen sowohl Risiko, wie auch Resilienzfaktoren für die Entwicklung chronischer Rückenschmerzen zu identifizieren. Das Ziel dieser Dissertation ist es, sowohl appetitive, wie auch aversive Lernmechanismen mit Hilfe funktioneller Magnetresonanztomographischer Aufnahmen in Patienten mit subakuten und chronischen Rückenschmerzen zu untersuchen und diese darüber hinaus mit einer gesunden Kontrollgruppe zu vergleichen. Schmerzpatienten zeigten maladaptive Veränderungen in emotionalen Lernprozessen, wobei das frühe subakute Schmerzstadium anders betroffen war als das chronische Rückenschmerzstadium. Diese Veränderungen zeigten sich in den subakuten Schmerzpatienten in einer schwächeren Aktivierung des Hippocampus und der Amygdala, aber auch durch eine stärkere Aktivierung des Operculums parietale, im Vergleich zu gesunden Kontrollprobanden während des appetitiven Lernens. Patienten mit chronischen Rückenschmerzen zeigten im Vergleich zu der Kontrollgruppe eine schwächere Aktivierung des Hippocampus und des Nucleus accumbens, neben einer gesteigerten Aktivierung im posterioren cingulären Cortex. Im Vergleich zu den Kontrollprobanden zeigten beide Schmerzpatientengruppen, während des appetitiven Lernens, eine Verlagerung der Gehirnaktivierung von belohnungsbezogenen zu schmerzbezogenen Gehirnregionen. Zudem weisten Patienten mit subakuten und chronischen Rückenschmerzen ein gesteigertes aversives Lernen auf, welches sich vornehmlich im limbischen System zeigte. Außerdem hat sich in der subakuten Patientengruppe gezeigt, dass die während des emotionalen Lernens evozierte Gehirnaktivität hauptsächlich durch die Verarbeitung sensorisch-affektiver Stimuli im orbitofrontalem Cortex angetrieben wurde. Der orbitofrontale Cortex ist eine Gehirnregion, welche funktionell der Belohnungsverarbeitung zugeordnet wird. Diese Gehirnregion beeinflusste das Lernverhalten in der subakuten Patientengruppe, unabhängig davon welche Lernmechanismen untersucht wurden. Die Ergebnisse dieser Dissertation suggerieren, dass emotionales Lernen ein essenzieller Mechanismus sein kann, der den Übergang von akuten zu chronischen Schmerzen bedingt. Dies zeigt sich in neuroplastischen Veränderungen im Gehirn, welche prädiktiv den Chronifizierungsprozess darstellen können.

Abstract

Chronic back pain is a worldwide health issue. Patients suffering from chronic pain often live in a vicious cycle of disability and distress. Neuroimaging studies have already shown that the brain changes in its structure and function once the pain has become chronic. However, it is not well understood, whether these changes are also predictors of pain chronicity and which are the relevant underlying mechanisms in this process. Emotional learning may play an important role in this context. The investigation of functional and structural changes in the brain concomitant with associated emotional learning mechanisms might help to identify risk and resilience factors in the transition from acute to chronic back pain. The aim of this thesis is to investigate appetitive and aversive learning mechanisms in patients with subacute back pain and chronic back pain, compared to a group of healthy controls, using functional magnetic resonance imaging. Emotional learning-related brain mechanisms seemed to be maladaptive in patients with back pain affecting the subacute and chronic back pain stage differently. This was indicated by a weaker activation of the hippocampus and the amygdala, but stronger activation in the parietal operculum during appetitive learning in subacute pain patients when compared to healthy controls. Chronic back pain patients showed weaker activations in the nucleus accumbens and the hippocampus besides stronger activation seen in the posterior cingulate cortex in comparison to the healthy control sample. Both pain samples showed a shift away from reward-related brain regions towards pain-related brain areas during appetitive learning. Subacute and chronic back pain patients revealed enhanced aversive learning responses in comparison to healthy controls with a strong impact of the limbic system on learning-related brain activation. Moreover, emotional learning responses in subacute back pain patients seemed to be driven by responses to affective sensory stimulation in the orbitofrontal cortex. A brain region which is thought to process reward-related information, such as the orbitofrontal cortex, influenced learning in the early subacute pain stage, irrespective of the tested learning mechanism. These findings suggest that emotional learning might be an important mechanism causing the transition from acute to chronic pain, indicated by neuroplastic changes in the brain that may serve as predictive markers of pain chronicity.

1. Introduction

Every human being will at least one time in his or her life suffer from musculoskeletal pain. The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al. 2020). Usually the pain disappears after some time, but sometimes it persists past its healing and becomes chronic. When pain becomes chronic, it loses its protective function of signalling a threat. Chronic pain is defined as pain persisting past its healing for more than 3 to 6 months (Apkarian, Sosa, Krauss, et al. 2004; Treede et al. 2015). Chronic musculoskeletal pain affects around 20 % of the worldwide population and is characterised by nociceptive pain affecting bone, muscle, joints or any related tissue (Treede et al. 2015). In most patients with chronic musculoskeletal pain, an underlying physiological cause is absent (Raspe, Hueppe, and Neuhauser 2007) and is therefore hard to treat (Apkarian, Hashmi, and Baliki 2011; Flor 2012; Hashmi et al. 2013; Hart, Martelli, and Zasler 2000). People with chronic musculoskeletal pain are burdened with their pain problem and often report a low quality of life, as their constant pain is stressful for their body, affects their activity levels and social interactions (Apkarian 2008; Baliki et al. 2006; Dueñas et al. 2016; Hart, Martelli, and Zasler 2000; Mouraux and Iannetti 2018). Patients with chronic pain often live in a vicious cycle of disability and suffering and show enhanced fear of pain and avoidance behaviours (Crombez et al. 2012; Vlaeyen et al. 1995). In addition, patients suffering from chronic pain seem to be more prone to develop mood and anxiety disorders (Apkarian 2008; Apkarian, Hashmi, and Baliki 2011; Baliki et al. 2006; Flor 2012; Mutso et al. 2014). The mechanisms by which the transition from acute to chronic pain is driven are, however, still not well known. In this thesis I will outline the potential critical and currently debated mechanisms and theories behind the chronification of pain. I will then interpret and discuss the preliminary results of my experimental work in this framework, including the neural correlates of such mechanisms.

1.1 Pain, fear avoidance and learning

Patients with chronic pain often live in a vicious cycle of disability and suffering, driven by a fear of pain which causes avoidance behaviours (Crombez et al. 2012; Vlaeyen et al. 1995). How the transition from acute to chronic pain might be driven was discussed in different theoretical models. A prevalent one in the context of fear learning and pain is the fear avoidance model (Lethem et al. 1983; Vlaeyen et al. 1995). According to this model, pain-related fear is the key element to drive the transition from acute to chronic pain. Depending on how subjects interpret their acute pain, they either recover from their pain problem or are trapped in a vicious cycle of disability and suffering (Crombez et al. 2012; Vlaeyen et al. 1995). Subjects, who interpret their pain as threatening and signalling an injury, will show a heightened fear of pain. This fear will result in a reduction of physical activities

and in hypervigilance to pain and pain-related information. In most subjects this will culminate in avoidance behaviours that interfere with their everyday-life, thereby reducing positive experiences and affecting their social life (Crombez et al. 2012; Vlaeyen et al. 1995; Vlaeyen and Linton 2000; Vlaeyen and Linton 2012). A decrease in physical activity (Hasenbring 1993) and an increase in negative emotional states (Kamping et al. 2013) can then exacerbate the pain that subjects experience, creating a self-reinforcing vicious cycle of chronic pain. In contrast to this, subjects who interpret their pain as non-threatening recover from it (Figure 1).

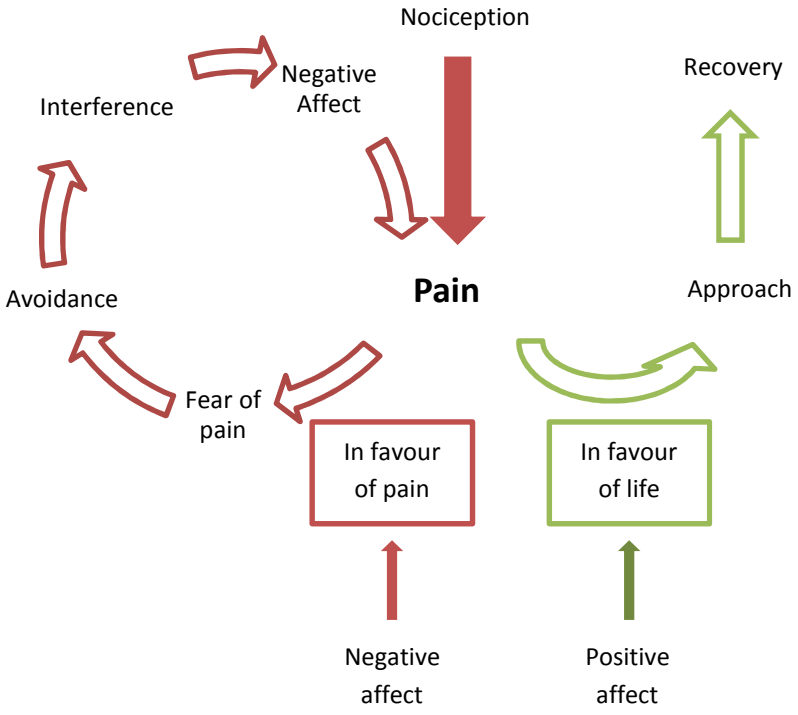

Figure 1: Fear avoidance model. A nociceptive input elicits pain. Depending on how subjects interpret their pain, they will either recover from it (right side) if they interpret their pain as non-threatening or enter the cycle of pain-related fear (left side) if they interpret their pain as threatening. The cycle of pain-related fear will end in chronic pain, causing avoidance behaviours, leading to interference with everyday life and negative affect (model adjusted from Vlaeyen et al. 2016).

Fear is an adaptive emotional response to a threat which can be experienced (being in an accident), observed (seeing a climber falling down a boulder and getting injured) or induced (e.g. by instructions like 'You will receive unbearable pain.') (Vlaeyen, Crombez, and Linton 2016). The fear avoidance model highlights the importance of emotional learning in the development of chronic pain, where a maladaptive emotional response is learnt. Learning pain-related fears influences the perception of pain itself, as well as neuropsychological and biochemical aspects of pain, which outlast the acute pain and thereby potentially contribute to chronic pain (Flor 2012). In line with this, emotional learning can drive the chronicity process. At first, acute pain will serve as an unconditioned stimulus (US). It will

be then associated with any other presumably innocuous somatosensory input, eliciting conditioned responses (CRs) like muscle tension (Moseley et al. 2003). These CRs can later on be evoked, even after the original acute pain is gone (Flor et al. 1992; Flor and Birbaumer 1994; Linton, Melin, and Götestam 1984; Moseley et al. 2003; Traxler et al. 2019). In line with the fear avoidance model, the importance of emotional learning was discussed to play a key role in the development and maintenance of chronic pain (Apkarian et al. 2005; Apkarian 2008; Apkarian, Baliki, and Geha 2009; Flor 2012; Nees and Becker 2018; Traxler et al. 2019; Zhang et al. 2019), especially of musculoskeletal pain. Pain-related fear is associated with maladaptive learning processes, such as enhanced fear generalization to novel innocuous stimuli (Meulders, Vansteenwegen, and Vlaeyen 2011; Moseley et al. 2003) resulting in pain memories which will then lead to the development and manifestation of pain behaviours (Flor 2000; Flor 2002; Flor 2012; Fordyce 1984). Once the pain-related fear has strengthened the path for the development of chronic pain is set, driving maladaptive learning mechanisms. The constant presence of pain makes it harder to extinguish those associations (Apkarian 2008; Flor 2000; Flor 2002; Flor, Knost, and Birbaumer 2002). In line with this concept, patients with higher pain-related fear were shown to over-estimate anticipated pain. In a study from McCracken et al. (1993), patients with lower back pain were asked to perform a straight lift of their leg and those with greater pain-related fear tended to stop leg raises earlier than less anxious patients (McCracken et al. 1993). Pain-related fear and avoidance causes hypervigilance to threat and an overestimation of future pain and its consequences in pain patients (Crombez et al. 2012; McCracken et al. 1993; Vlaeyen and Linton 2000). Heightened pain-related fear was discussed to result in negative appraisal about pain and in avoidance behaviours interfering with daily life activities. Avoidance behaviours will occur in expectation of pain rather than as a consequence of pain, whereby patients have fewer opportunities to correct their wrongly made expectations when the initial pain is gone (Vlaeyen and Linton 2000; Vlaeyen et al. 1995). Furthermore, it was discussed that in patients with chronic pain the valuation of pain and reward is biased towards pain avoidance and pain relief (Nees and Becker 2018).

1.2 Learning paradigms in pain research

Emotional learning can be studied using classical conditioning paradigms. Classical pavlovian conditioning consists of the acquisition of a conditioned response (CR) which is elicited after the presentation of a beforehand neutral stimulus. A neutral stimulus (e.g. geometrical figure, colour, face) is used as a conditioned stimulus (CS) which is paired to the presentation of an aversive or appetitive stimulus (unconditioned stimulus, US). The presentation of the US alone will produce an unconditioned response (UR), such as flinching after a painful electrical stimulation. After the association between the presentation of the neutral CS and the US is established, the presentation of the CS alone will elicit a similar reaction as the UR which will then become the CR. The CS

presentation alone will produce the CR, since it has acquired emotional significance (Dunsmoor et al. 2014; Pavlov 1927). During extinction, the CS is then repeatedly presented alone, leading to a decline in the previously established CR (Nees, Heinrich, and Flor 2015). Classical conditioning procedures often include differential conditioning paradigms in which two neutral CSs are presented: the CS+ will be coupled to the presentation of the US, whereas the CS- is never paired to the US (Cacciaglia et al. 2015; Martin-Soelch, Linthicum, and Ernst 2007; Pohlack et al. 2012; Sehlmeyer et al. 2009). In addition, CS+ trials can be only partially reinforced by the US presentation, for example only 50 % of the CS+ presentations will be paired to the US (CS+ paired), whereas the rest of the CS+ trials (CS+ unpaired) will be presented alone (Cacciaglia et al. 2015; Pohlack et al. 2012). Differential conditioning gives researchers the opportunity to analyse underlying brain responses which can be considered as learning-related brain responses rather than activations due to the processing of the US. Further, differential conditioning helps to identify responses during danger signals (aversive CS+) and safety signals (CS-/ appetitive CS). In addition, it controls for non-dependent learning processes, such as dishabituation or sensitization during conditioning (Nees, Heinrich, and Flor 2015). Reinforcement rates can differ depending on the research question and tested sample (Sehlmeyer et al. 2009). The type of the used US allows to compare between different learning mechanisms, such as aversive and appetitive learning. Data on conditioning in chronic pain are sparse. In patients with chronic back pain (CBP) enhanced pain perception, faster acquisition of fear responses and slower extinction of fear was found when compared to healthy controls (HC) (Diesch and Flor 2007; Meulders, Vansteenwegen, and Vlaeyen 2011; Schneider, Palomba, and Flor 2004). Further, patients with CBP showed exaggerated muscular responses (Flor, Knost, and Birbaumer 2002; Meulders, Vansteenwegen, and Vlaeyen 2011; Schneider, Palomba, and Flor 2004) evoked by fear of pain and avoidance behaviours after aversive conditioning with a painful US (Traxler et al. 2019). Non-differential fear generalization to a novel experimental context was seen in fibromyalgia patients, whereas HC showed fear of movement-related pain to movements resembling the original painful one (Meulders, Jans, and Vlaeyen 2015).

A lot of research has focused on aversive learning and the involvement of the underlying brain circuits. Only a minor portion of studies focused on appetitive learning. Appetitive learning describes the process of learning associations between rewards and neutral stimuli or behaviours (Martin-Soelch, Linthicum, and Ernst 2007). For successful learning the value/salience of the US has to be high/positive enough. The assessment of safety signals is equally important as the assessment of danger signals (Nees, Heinrich, and Flor 2015). It was shown that patients with chronic pain perceive positive stimuli, such as a pleasant touch (PT), as less pleasant than controls (Nees and Becker 2018; Nees et al. 2019). Compared to HC, the neuronal processing of the PT was altered in patients with CBP and changed as a function of back pain duration (Nees et al. 2018). Fibromyalgia patients with intact sensitivity for affective touch, reported slow brushing movements as less pleasant than HC, which was reflected in a decreased activation in the insular cortex (IC) during pleasantness ratings

(Boehme et al. 2020). A PT can influence the evaluation of a previously harmful touch and can turn it into a pleasant stimulus (Löken, Evert, and Wessberg 2011). Kamping and colleagues (2013) showed that fibromyalgia patients, but not controls, showed impaired pain inhibition when viewing positive affective pictures which was associated with changes in brain activation in the secondary somatosensory cortex (S2), IC, orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) (Kamping et al. 2013). These results highlight the importance of appetitive learning also in chronic pain. The findings further indicate a specific role of aversive and appetitive learning in the development and/or maintenance of CBP. Appetitive learning could have a strong impact, especially on the transitional state. Understanding the mechanisms of emotional learning could pave the path for therapeutically relevant interventions.

Furthermore, changes in emotional learning affected the underlying brain circuits. Brain areas which are pivotal in learning and memory, such as the ACC, IC, hippocampus (Hipp), amygdala (Amy) (Büchel et al. 1998; Sehlmeyer et al. 2009; Seymour et al. 2005), but also the thalamus (Tha), medial prefrontal cortex (mPFC) and the striatum (Dunsmoor and LaBar 2012; Mutso et al. 2012; Sehlmeyer et al. 2009) were also reported to be involved in pain processing and more interestingly also in chronic pain (Apkarian 2008). Moreover, in chronic pain these brain correlates were reported to be altered (Mutso et al. 2012). Patients with chronic pain showed cognitive impairments, such as deficits in learning and memory. This was specifically observed in patients with CBP or complex regional pain syndrome. Animal models of pain indicated that cognitive impairments are likely driven by several chemical and cellular neuromodulators whose expression and/or activation levels were altered in the respective model of chronic pain (Moriarty, McGuire, and Finn 2011).

1.3 Brain correlates of pain

More than two decades of intensive research have been dedicated to the topic of chronic pain which has led to many intriguing results. For example, acute pain activates brain areas involved in nociception and saliency (Hashmi et al. 2013; Mouraux and Iannetti 2009; Mouraux et al. 2011), such as the IC, the ACC, the Tha, the primary somatosensory (S1) and S2 (Apkarian et al. 2005; Apkarian 2008; Mouraux et al. 2011). Chronic pain engages brain areas involved in cognitions and emotions, as suggested by a strong activation of the prefrontal cortex (PFC) (Apkarian et al. 2005; Apkarian 2008). Moreover, changes in functional connectivity have been reported in CBP, including the default mode network (Apkarian, Hashmi, and Baliki 2011; Baliki et al. 2006; Baliki et al. 2010; Baliki et al. 2012; Farmer et al. 2011), the connectivity between mPFC and the Hipp (Mutso et al. 2014), the mPFC and nucleus accumbens (NAC) (Baliki et al. 2006) and between the putative shell and core of the NAC (Makary et al. 2020). The last two were assigned to have a predictive power whether patients with subacute back pain (SABP) will develop CBP. Furthermore, apart from these functional changes,

structural changes in grey matter density have also been reported (Baliki et al. 2006; Baliki et al. 2011) with a decrease in hippocampal volume (Mutso et al. 2012), the Amy (Vachon-Presseau, Tetreault, et al. 2016), the dorsolateral prefrontal cortex (dlPFC) and the right Tha which could be related to the pain characteristics and pain duration (Apkarian, Sosa, Sonty, et al. 2004; Baliki et al. 2012). These findings are supported by the idea that the pain experience itself drives functional changes in synapses, resulting in structural synaptic alterations which create long-term memory traces (Farmer et al. 2011), thereby affecting decreases in grey matter via long term depression. Furthermore, structural and functional changes in chronic pain were often reported to affect the limbic system (Flor 2012; Hashmi et al. 2013; Nees and Becker 2018). The limbic system is important for the coding of emotion, cognition, behaviour, motivation and memory functions (Bushnell, Ceko, and Low 2013; Nees and Becker 2018), as well as in reward processing and related behaviours (Nees and Becker 2018). It is composed of the striatum, mPFC, Hipp and Amy (Vachon-Presseau, Tetreault, et al. 2016). A longitudinal study comparing brain activation patterns of chronic and acute pain could show that chronic pain activated brain areas which are known to be involved in processing of emotions, whereas acute pain activated pain-related brain areas (Hashmi et al. 2013). SABP patients with persisting pain after one year showed a shift towards these emotion-related brain areas, similar to brain activation patterns already observed in the CBP group (Hashmi et al. 2013).

The beforehand described behavioural, functional and structural brain changes in chronic pain are so far lacking a causal relationship. It is still not well understood what is causing the transition from acute to chronic pain. Here, emotional learning mechanisms involving aversive, as well as appetitive learning mechanisms seem to be the key to affect the transitional state. There is a high overlap between reported brain changes in CBP, emotion processing and in learning-related brain areas, stressing the fact that emotional learning is maladaptive in chronic pain. So far it is not clear whether altered learning mechanisms lead to chronic pain or to what extent the chronicity affects learning.

1.4 Study goals

The aim of this thesis is to investigate appetitive and aversive learning in different pain populations and to test whether learning becomes maladaptive with pain persistence when compared to a group of HC. Brain responses during learning are investigated in a cross-sectional manner, comparing brain activation maps, as well as physiological data (skin conductance) and behavioural ratings between HC, patients with SABP and CBP using functional magnetic resonance imaging (fMRI) data during conditioning. Patients with SABP pain are in their first pain episode, therefore reflecting the group at high risk to develop CBP or to show resilience. SABP patients are able to bridge the gap between being pain-free and suffering from CBP, thereby helping to get a better understanding of the transitional state in the development of chronic pain. To test aversive and appetitive learning

mechanisms in the context of chronic pain is equally important, because lacking the ability to gain similar pleasure from positive events is equally important as avoiding painful situations. In order to get a better understanding of emotional learning mechanisms and to identify its underlying brain circuits, these mechanisms need to be investigated and compared. At first, I investigate emotional learning mechanisms in SABP and CBP patients and analyse whether appetitive and aversive learning are equally altered or whether changes in emotional learning are a consequence of the chronicity process. Moreover, I explore if pain patients have deficits in sensory processing and whether processing of either painful or pleasant stimuli is altered.

1.5 Hypothesis

1. I hypothesize that patients with CBP will show impaired acquisition of appetitive learning.
2. I assume that pain patients will reveal heightened learning responses during aversive learning.
3. I propose that already the processing of the affective sensory stimuli is impaired in patients with CBP and those who are at high risk to develop chronic pain, leading to maladaptation in learning mechanisms:
    a. with a shift away from brain regions activated during reward processing during appetitive learning
    b. and stronger activation in emotion-related brain areas during aversive learning.
4. Lastly, I hypothesize that SABP patients are a heterogeneous group representing a continuum between HC and CBP patients, exhibiting brain activation maps with some shared similarities with both groups.

2. Material and Methods

2.1 Participants

Thirty-nine HC (15 female, mean age (M) = 34.61 ± standard deviation (SD) = 14.26), fifty patients with SABP (32 female, M = 39.68 ± SD = 15.11) and thirty-nine patients with CBP (16 female, M = 39.38 ± SD = 15.01) were included into the study sample (Table 2 = Tab. 2). All subjects are part of an ongoing longitudinal study of the CRC 1158 of the Heidelberg Pain Consortium. Inclusion criteria for both pain groups included localized pain in upper and/or lower back. HC had to be pain free. Exclusion criteria for all three subject groups included: neurological or sensory deficits, physical illnesses, left handedness, tinnitus, migraine, epilepsy, inflammatory pain, open-heart surgery, head surgery, implants (metallic), pacemaker, microblading, facial tattoos, schizophrenia, current drug abuse, psychotic episodes and pregnancy. In addition, exclusion criteria for both pain groups included any reported pain which was more severe than their back pain. HC with any past or present mental disorders, personality disorders, past or present pain that lasted longer than 3 month and magnetic resonance imaging (MRI) incompatibility, were excluded from the study. A psychologist interviewed all subjects performing the German version of the Structured Clinical Interviews (SCID) (Wittchen et al. 1997) for DSM IV. Mental illnesses such as anxiety and depression were excluded from the HC sample, but not from the pain patient samples, since comorbidity's are known to be common in chronic pain patients (Tunks, Crook, and Weir 2008; Gorczyca, Filip, and Walczak 2013). Any exclusion would have resulted in a biased study sample including only subjects with a lesser burdened course of disease. Equally, any medication was excluded from the HC sample, but not from the pain groups. All participants were right-handed, except for two SABP patients which were left-handed. Those two patients were included, due to difficulties in finding patients with SABP. In those two cases, fMRI data were tested for laterally shifted brain activation patterns in contrast to the right-handed subjects. In one subject data were vertically flipped to fit the right-handed brain patterns in both conditioning paradigms. Subjects gave their written informed consent and the study was approved by the Ethics Committee II of the Medical Faculty Mannheim, University of Heidelberg, Germany.

2.1.1 Pain patient groups

Pain patients (SABP and CBP) had either low and/or upper back pain (Tab. 1). The group of CBP patients was defined by any back pain episode which lasted longer than six months, with a minimum three days of existing pain per week. Also patients with slipped disks were included. SABP was defined as pain persistence for seven up to twelve weeks but no longer (Chanda et al. 2011). Patients who reported reoccurring pain for several years which did not exceed more than ninety pain days per year, were also included into the SABP sample. Pain had to be present at least during the last week in

both pain groups (SABP and CBP) for patients to be included into the study. Patients with neuropathic pain or any neurological or sensory abnormalities were excluded from all groups. Pain patients taking any medication or with diagnoses of any present or past psychological comorbidities, such as depression and anxiety, were not excluded from the study sample. Medication and comorbidities were carefully assessed. On each measurement day it was imposed whether subjects were suffering from back pain and its intensity on a scale from 0 (no pain) until 10 (worst pain imaginable). Pain intensity scales were collected to explain possible differences between subjects.

group	***HC***	***SABP***	***CBP***
Inclusion criteria	Age: 18-70 years, right-handed		
	pain free	upper and/or lower back pain	
Exclusion criteria	open-heart surgery, head surgery, implants (metallic) and pacemaker, epilepsy, microblading, facial tattoos, schizophrenia, current drug abuse, psychotic episodes, pregnancy, neurological deficits, tinnitus, left-handedness		
	any past or present pain problem, psychological disorders	inflammatory pain, acute borreliosis, neck pain, pain problem more severe than back pain	
Pain duration	pain free	7-12 weeks acute back pain OR recurrent pain < 12 weeks per year	minimum 12 weeks back pain within last 12 months

Table 1: Inclusion and exclusion criteria for study samples. Healthy controls were not allowed to report any past or present chronic pain, physical or mental disorder. Back pain patient groups were defined based on the duration of pain persistence and frequency of pain days per year. Subjects with less than 90 days of back pain per year were defined as subacute back pain and pain persistence for more than 90 days per year were defined as chronic back pain. It was not distinguished between lower and/or upper back pain, except if neck pain was the most severe pain. Patients, who reported any pain that was more severe or comparable to their back pain, were excluded. Group abbreviations: HC = healthy controls, SABP = subacute back pain, CBP = chronic back pain.

2.1.2 Study sample

Twenty-three subjects were excluded from the final analyses (13 appetitive conditioning = APP, 10 aversive conditioning = AVC) shown in this thesis. Subjects that showed outliers in the fMRI data with values more than 4 SD above or below the respective mean of the percentage blood-oxygen-dependent (BOLD) signal change, were discarded from the data-set (APP: 1 HC; 1 CBP, AVC: 1 HC, 2 CBP), also subjects that showed strong movements exceeding more than 3 mm (voxel size 3 mm, AVC: 1 SABP) and/or subjects that showed strong and significant activation of white matter tracts or in the ventricles, but not in the grey matter were excluded from the data analyses (APP: 1 HC, 3 SABP, 2 CBP; AVC: 1 HC, 1 SABP, 3 CBP). Furthermore, there were participants

who dropped out of the study, due to personal reasons (APP: 1 HC, 3 CBP) or subjects which were MRI incompatible (APP /AVC: 1 SABP) and were still assessed in the lab measurements, but were not included this thesis. Since both conditioning paradigms were tested on different measurement days (at least one week apart), sample sizes differ between the aversive and appetitive conditioning data. The aversive conditioning was always done on the first measurement day, due to other experiments of the overall project (which are not part of this thesis). Details of sample size numbers can be found in Tab. 2.

Appetitive Conditioning	*HC*	*SABP*	*CBP*	Aversive Conditioning	*HC*	*SABP*	*CBP*
Sample size (N)	39	50	39	**Sample size (N)**	39	50	39
Excluded subjects	3	4	6	**Excluded subjects**	2	3	5
Age (M±SD)	34.61 ± 14.26	39.68 ± 15.11	39.38 ± 15.01	**Age (M±SD)**	34.11 ± 13.89	40.19 ± 14.91	38.68 ± 14.74
Female	15	31	16	**Female**	15	31	17
Male	21	15	17	**Male**	22	16	17

Table 2: Study sample description. Data set used for the appetitive conditioning is depicted on the left hand side. Study sample used for the analyses of the aversive conditioning is depicted on the right side. Exclusion criteria for the analysed sample included general dropouts, MRI incompatibility, brain responses which deviated more than 4 SD percentage blood-oxygen-dependent (BOLD) signal change from the group mean, strong brain activation in the white matter tracts or in the ventricles, but not in the grey matter. Age ± M = mean, SD = standard deviation.

2.2 Experimental procedure

Emotional learning and its underlying brain mechanisms in the development of CBP was investigated using an aversive and an appetitive respondent conditioning paradigm (section 2.2.3) during fMRI. Both learning paradigms were tested in in a group of HC, patients with SABP and CBP (Tab. 1 and Tab. 2). Differential delay conditioning paradigms with a partial reinforcement rate of 50 % were used. Habituation (HAB), acquisition (ACQ1 and ACQ2) and extinction (EXT) were employed. Momentary pain status of each subject was retrieved before each measurement, on a scale from 0 (non-painful) to 10 (highest imaginable pain). The acquisition of aversive and appetitive learning was separated onto different sessions which were at least one week apart to prevent learning being influenced by the first learning experiment. Subjects were not informed about any similarities in the experiments. For stimulus delivery Presentation® software (Version 18.3, http://www.neurobs.com/) was used. Visual stimuli and the rating procedure were presented to the subjects via googles. Subjects with a deficient eyesight used lenses fitting their needs to compensate any problems in visual sight.

2.2.1 Behavioural and physiological assessments

After each of the four conditioning phases, behavioural assessments of the subjective valence, arousal and contingency awareness of the used CS and US was imposed with the help of visual analogue scales and Self-Assessment Manikins (Bradley and Lang 1994). Contingency awareness for the coupling of the CSs with the US was assessed using a nine-point Likert scale from 1 (very unlikely) to 9 (very likely), to assess whether subjects learned the contingencies between CS+ (stimulus which is paired to the US in 50 % of the trials) and the US, as well as the never paired CS-. Coupling between the US and the CS+ paired (CS+ pa.) was only employed during both acquisitions (ACQ1 and ACQ2), but contingency awareness was assessed over all phases. All subjects were trained beforehand to conduct the rating procedure on a MRI compatible keyboard, to ensure a reliable behavioural assessment without further interaction with the investigator. In addition, physiological measures such as the cardiac responses and respiration data were acquired with a built-in pulsoxymeter and a respiration belt during fMRI scans.

2.2.2 Skin conductance responses

Skin conductance responses (SCRs) were recorded during each conditioning phase using a BrainAmp ExG amplifier in combination with a GSR MR module (Brain Products GmbH, Gilching, Germany) at a sampling rate of 16 Hz. A constant current of 0.5 V was passed through bipolar 5 mm Ag/AgCl electrodes placed on the muscle abductor halluces of the left foot. Electrode cups were filled with an electrolyte gel (Brain Products GmbH, Gilching, Germany) before attachment. The recording procedure followed previously published guidelines (Boucsein 2012). SCRs were analysed using the Ledalab software (Version V3.4.9, http://www.ledalab.de/) package for Matlab. Continuous Decomposition Analysis (CDA) was used to analyse the event-related data (Benedek and Kaernbach 2010). SCR amplitudes were defined as the maximum response amplitudes in the time window of 1-12 seconds (sec) after the CS onset with a criterion of the smallest recordable SCR set at 0.01 micro Siemens (μS). This time window was chosen, because largest SCRs were found in this timeframe in the inspection of the raw data. SCRs were log transformed (log10) and averaged across trials, respectively, for the used stimuli in the experiment (CS+ unpaired, CS+ pa. and CS-). Group SCR results were calculated by averaging mean SCRs for each participant within each group. Differential SCRs were obtained by subtracting the SCRs elicited by the CS- from those triggered by the CS+ unpaired (CS+ un.). Group comparisons were estimated using common statistical tests (section 2.4.4). Due to methodological reasons and non-responsiveness of participants, data of forty-seven subjects had to be excluded from the appetitive conditioning data and forty data-sets from the aversive conditioning data (APP: 15 HC, 16 SABP, 16 CBP; AVC: 12 HC, 14 SABP, 14 CBP).

2.2.3 Learning paradigms

Aversive conditioning

A well-established differential fear-conditioning paradigm consisting of four phases (Figure 2) during fMRI was used to test aversive learning (Cacciaglia et al. 2015; Pohlack et al. 2015; Winkelmann et al. 2016). Two grey geometric figures, different in shape (circle and triangle) as depicted in Figure 2 (Fig. 2), were used as CSs. As US, a painful but tolerable electrical stimulation to the left thumb was used, by using a cupric electrode connected to a constant current stimulator (model DS7A, Digitimer, Hertfordshire, United Kingdom). Thresholds for the electrical stimulation were estimated for each subject, respectively, using an ascending staircase evaluation of perception (Levitt 1971), consisting of the sensation threshold (first perception of the stimulus), pain threshold (first painful perception) and the tolerance threshold (unbearable pain). The procedure was repeated three times. Pain thresholds and tolerance thresholds were calculated independently by averaging. Stimulus intensity was chosen to reach 80 % of the absolute pain tolerance level on the painfulness and unpleasantness scale. The stimulus was rated on a scale from 0 (non-painful) to 10 (highest imaginable pain). Pain intensity was increased until the desired painfulness and unpleasantness rating was reached (rating value of 8). Subjects were informed about the chosen stimulus. The CS+ was coupled in 50 % of the trials during both acquisitions to the US, whereas the CS- was never coupled to the US. During HAB CS+, CS- and the US were presented six times, respectively. The US stimulation was applied during the presentation of a black screen with a cross hair at the centre of the screen. During ACQ1 and ACQ2, CS+ and CS- were presented ten times each, (5 CS+ pa.: CS+ coupled to the US, 5 CS+ un.: CS+ presented alone, CS-: was never paired to the US). Both CSs were presented eight times, respectively, during EXT and US stimulations were stopped. The CSs were presented for 5.8 sec and the US for 2.8 sec, both stimuli co-terminated in CS+ trials where coupling occurred (CS+ pa.). US presentation started 3 sec after the onset of CS+ pa. presentation. Inter-trial-intervals (ITI) ranged from 7-12 sec in which a fixation cross was presented at the centre of the screen. Stimuli were displayed in a pseudo-random order. Subjects were not informed about contingencies between the CS+ and the US. All participants were instructed that visual stimuli are presented and that they will receive electrical stimulations (US) at the left thumb.

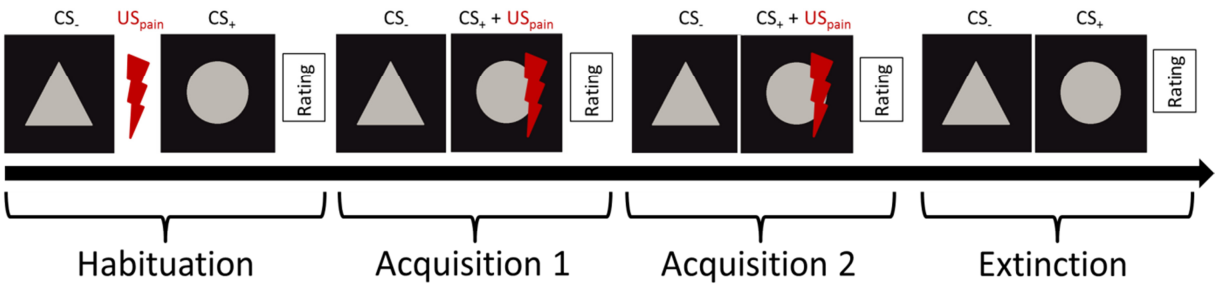

Figure 2: Experimental design of the aversive conditioning paradigm used during fMRI. The experiment consists of four phases, each followed by a subjective assessment of arousal, valence and contingency (Rating) of the used stimuli (CS = conditioned stimulus, US = unconditioned stimulus). Stimuli are presented in a pseudo random order. The CS+ is coupled to the presentation of the US (painful electrical stimulation to the left thumb) in 50 % of the CS+ trials during acquisitions. The CS- is never coupled to the US. CS+, CS- and the US are presented six times, respectively, during the habituation (HAB). The US is applied during a presentation of a black screen with a cross-hair. Inter-trial-intervals (ITI) consisted of a black screen with a cross hair in the middle of the screen. ITI duration ranged between 7-12 seconds (sec). During acquisition 1 and 2 (ACQ1 and ACQ2), CS+ and CS- are presented ten times each, five trials of the CS+ presentation are coupled to the US presentation (CS+ paired). In the extinction (EXT) CS+ and CS- are both presented eight times without any coupling to the US.

Appetitive conditioning

To ensure comparability of the data and to investigate the underlying brain patterns during the processing of either aversive or appetitive stimuli, the same conditioning paradigm was used for the appetitive learning, as during aversive learning. Visual cues (CSs) and the US were exchanged to create a new experiment out of the subjects' perspective. Instructions were given in the same manner as on the first measurement day, but adjusted to the appetitive conditioning paradigm. A PT was used for the touch stimulation, serving as an appetitive US in the learning experiment. The PT was a MR-compatible robotic touch-stimulator, consisting of a small cosmetic brush attached to a small robotic arm which was mounted onto a plastic board. It was comfortably stabilized on the subjects' chest to deliver brush strokes to the subjects' left forearm. Stroking movement was unilateral starting proximal to distal with a velocity of 3 cm/sec (e.g. Nees et al., 2018). Experimental design can be seen in Fig. 3.

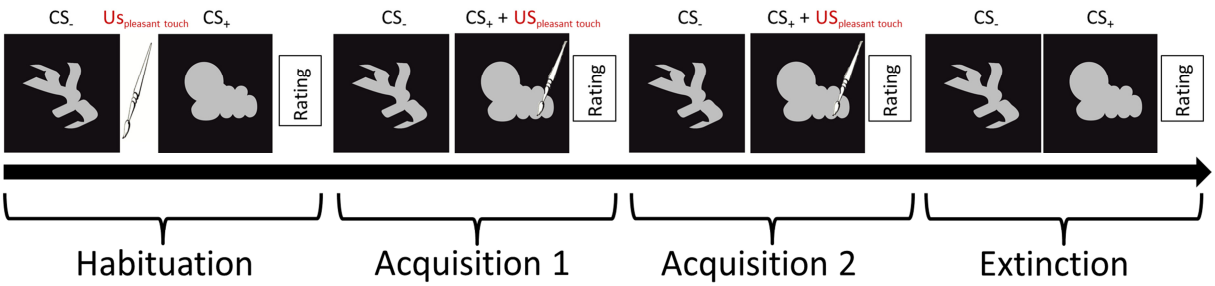

Figure 3: Experimental design of the appetitive conditioning paradigm used during fMRI. The experiment consists of four phases, each followed by a subjective assessment of arousal, valence and contingency (Rating) of the used stimuli. Stimuli are presented in a pseudo random order. The CS+ is coupled to the presentation of the US in 50 % of the CS+ trials during acquisitions. The CS- is never coupled to the US. CS+, CS- and the US are presented six times, respectively, during the HAB. The US is applied during a presentation of a black screen with a cross-hair. ITI consisted of a black screen with a cross hair in the middle of the screen. ITI duration ranged from 7-12 sec. During ACQ1 and ACQ2, CS+ and CS- are presented ten times each, five trials of the CS+ presentation are coupled to the US presentation (CS+ paired). In the EXT CS+ and CS- are both presented eight times without any coupling to the US. A stroke with a MRI compatible pleasant touch (PT) is used as a bodily related US. Two grey abstract figures, different in shape, serve as CSs. Experimental setup is similar to the aversive conditioning with exchanged US and CSs to ensure comparability between the two paradigms.

2.3 Magnetic resonance imaging

2.3.1 Structural magnetic resonance imaging

MRI data were acquired on a 3 Tesla MAGNETOM Trio whole body scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard 12-channel head coil using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence, for the anatomical scan with the following settings: repetition time (TR) = 2300 ms, echo time (TE) = 2.98 ms, field of view (FoV) = 240 mm * 256 mm, 192 sagittal slices, voxel size = 1.0 mm isotropic, flip angel (α) = 9°, parallel imaging (GRAPPA) factor. The MPRAGE was used for both conditioning paradigms.

2.3.2 Functional magnetic resonance imaging

Whole-brain fMRI data were acquired using a T2*-weighted gradient-echo echo-planar imaging (EPI) sequence: TR = 2350 ms, TE = 22 ms, FoV = 220 mm * 220 mm, 40 contiguous axial slices, slice thickness = 2.3 mm, voxel size = 2.3 mm isotropic, α = 90° with GRAPPA-technique acceleration factor 2. To account for inhomogeneity in the magnetic field, a shim was done before the acquisition of the data. In addition, a standard gradient field map was recorded.

2.4 Data analyses

In all analyses, focus was laid on the BOLD responses in the three subject samples during ACQ1 and ACQ2. BOLD responses for all presented stimuli were examined separately within each group. In addition, learning-related brain activation patterns were investigated. To analyse learning-related brain activation, results of the CS+ un. trials were compared with the CS- trials. BOLD responses which were greater in CS+ un. trials than in the CS- trials (CS+ un. > CS-) were used for the learning contrast, within each group, respectively. Additionally, learning-related brain responses were compared between subject samples (group comparison). Different procedures were used to analyse the fMRI data, such as whole-brain analyses and region of interest (ROI) analyses which will be further discussed in the following sections. FMRI data of the HAB and EXT are not included in this thesis. Behavioural and SCR data can be found in the supplement (suppl., section 6).

2.4.1 Preprocessing whole-brain fMRI analyses

For the whole-brain analyses of the structural scans and the fMRI data, a combination of SPM8 (Statistical Parametric Mapping Welcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, UK) and FSL (FMRIB Software FMRIB Software Library v5.0, Analysis Group, FMRIB, Oxford, United Kingdom) was used. In a first step, within-subject data (first level) were analysed. Several preprocessing steps were used to prepare the data. The first three scans of all fMRI measurements were excluded to account for any distortions in the magnetic field. In addition, a field map correction was applied to undistort the magnetic field and to improve spatial accuracy of the EPI sequences of both conditioning paradigms. All non-brain structures were removed from the EPI scans using the FSL Brain extraction tool (BET) (Smith 2002). The T1-weigthed anatomical image of each participant was extracted using the SPM8 voxel-based morphometry tool (VBM8) to segment grey matter, white matter and the cerebrospinal fluid (CSF). All following processing steps (preprocessing, first level, higher level) were carried out in FSL using its FMRI Expert Analysis Tool (FEAT). Preprocessing steps included motion correction using FMRIB's Linear Image Registration Tool MCFLIRT (Jenkinson et al. 2002), distortion correction (B0 unwarping), high-pass temporal filtering with a 100 sec cut-off to remove low-frequency drifts, slice-time correction (regular down) and spatial smoothing using an isotropic 5 mm Gaussian kernel of full-width at half maximum. All scans were realigned to the fourth scan of each EPI sequence (slice timing correction). Co-registration of the EPI images was done sequentially. In a first step, EPI scans were registered to the individuals` brain extracted MPRAGE using FMRIB's FLIRT (Jenkinson et al. 2002; Jenkinson and Smith 2001). In a second step, the resulting image was registered non-linearly (FNIRT) to a standard brain in Montreal Neurological Institute (MNI) 152 space (Andersson JL 2007a, 2007b).

2.4.2 First level and higher level analyses

Resulting time series data were analysed using a general linear model (GLM) approach. CS+ un., CS+ pa., CS- and US trials were modelled as separate parameters of interest (regressors) and were convolved with the hemodynamic response function (Double-Gamma HRF) in the GLM, as to test their evoked activation patterns. Furthermore, the main parameter of interest was always included to test for learning-related BOLD responses by contrasting the CS+ un. trials > CS- trials. Six additional motion parameters of no interest were included, to model rotation and translation of rigid body movements. Significant responses were identified using a cluster correction threshold $Z > 2.3$ at a significance level of $p < 0.05$. After calculating subject means utilizing fixed effect analysis, higher level analyses were used to estimate within-group effects and between-group effects for the learning regressor (CS+ un. > CS-) using FMRIB's Local Analysis of Mixed Effects (FLAME). FLAME models and estimates the random-effects component of the measured intersession mixed-effects variance for group statistics. Images were cluster corrected with a Z-score at 2.3 and a significance level of $p < 0.05$. To test for group differences, results of the learning contrast were tested statistically between groups (section 2.4.4). Data of BOLD responses during HAB and EXT were not included in this thesis.

2.4.3 Region of Interest analyses

Additionally to the whole-brain analyses, ten independent regions of interest (ROI) were defined: Hipp, Amy, IC, mPFC, dlPFC, OFC, ACC, S1, S2 and the NAC. ROI masks were based on the FSL Harvard-Oxford cortical and subcortical structural atlases (Eickhoff 2007), to get a better understanding of the involvement of different brain areas in both learning paradigms and how those differ between the three subject groups. The masks for the ROI analyses were defined by either using standard masks of the structural Harvard-Oxford atlases or were defined based on findings in the literature. A sphere of 8 mm was built around the given MNI coordinates for the respective brain area. Masks of the mPFC and the dlPFC were created based on the work of Baliki and colleagues (Baliki et al. 2012) who had a similar research focus in their studies, investigating functional brain changes in the development of CBP. Peak coordinates for the mPFC were chosen as MNI coordinates: $x = 2$, $y = 52$, $z = -2$ for the left mPFC which was then merged with the mask of the right mPFC ($x = -2$, $y = 52$, $z = 2$) by using the fslmaths command. Resulting in a mask for the mPFC with MNI coordinates: $x = 0$, $y = 52$, $z = 0$. A similar procedure was used to create the mask for the dlPFC covering both sites of each brain hemisphere with a diameter of 8 mm (left side: $x = 44$, $y = 26$, $z = 32$ and right side $x = -44$, $y = 26$, $z = 32$). The Harvard-Oxford mask of the ACC was reduced in its size since the probabilistic mask covered the whole cingulate gyrus. The size of the ACC mask was reduced to include only parts of the mask in which a probability threshold of 30 % was reached. All masks were binarized before the ROI analyses, achieving that voxels within a mask had a value of 1

and outside the mask a value of 0. The percent bold signal change (BSC) was extracted using the FSL featquery tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) from each ROI in the learning trials and for each group, respectively. For this purpose, Harvard-Oxford masks were put into the participant's native space creating individual spherical masks (8 mm) around the peak voxel. ROI analyses reported here were always done in masks covering both sites of the respective brain area (exception mPFC).

2.4.4 Statistical Analyses

Statistical analyses were performed using RStudio 1.2.5001 (RStudio, Inc., Boston, MA) with R 3.4.0 (The R Foundation for Statistical Computing), Statistical Package for Social Sciences (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and MATLAB R2016a (© 1994-2018 The MathWorks, Inc.). The analyses of the behavioural assessments of arousal, valence and contingency awareness of both conditioning paradigms, as well as the SCR were processed similarly. In a first step, responses for the CS+ and CS- were statistically compared using an analysis of variance (ANOVA) with group as a between factor. This was followed by comparisons of the respective group means (pairwise t-test or Wilcoxon rank sum test), corrected for multiple testing by controlling the false discovery rate (FDR, (Benjamini and Hochberg 1995) to test for within group effects of the CS+ versus CS- values, respectively, for SCR, arousal, valence and contingency ratings. Furthermore, group differences were calculated using the same approach comparing Delta CS results (CS+ un. minus CS-) across groups, applying an unpaired post-hoc t-test (FDR corrected) to compare group means between two groups.

2.4.5 Comparison of fMRI data and behavioural responses

Correlational analyses were carried out between the BSC during learning in the eight ROIs and the behavioural valence ratings for the US (HAB) in both paradigms. A Shapiro-Wilk test was performed to test for normal distribution of the data. If data were not normally distributed, Spearman's Rank correlation coefficient (Spearman's rho) was computed, else Pearson correlation coefficient was calculated (Pearson's rho). The threshold for significance of rho was set at $p < 0.05$.

2.4.6 Correlation of pleasant touch/painful stimulation and learning-related responses

A linear regression was computed using the Rstudio stargazer package (Hlavac, Marek; 2018, Well-Formatted Regression and Summary Statistics Tables, R package version 5.2.1). The linear regression model was used to test the impact of initial BOLD responses during appetitive/aversive sensory stimulation during the HAB on the learning-related brain responses during both acquisitions. Before actual performance of the linear regression, data were tested whether they fulfilled the requirements for a linear regression (normality, multicollinearity, auto-correlation, homoscedasticity)

using SPSS built in formulas. Data fulfilled most of the given criteria, but some data showed auto-correlation. The BOLD responses of the PT stimulation during HAB were used as the dependent variable, for the regression model and the learning-related brain responses as the independent variable, within each ROI, respectively, per group.

3. Results

The objective of this thesis is to investigate appetitive and aversive learning mechanisms and their maladaptation in different back pain populations. FMRI data which were acquired during an appetitive and an aversive conditioning paradigm were analysed in a group of HC, patients with SABP and CBP. Behavioural assessments of valence, arousal and contingency ratings were aquired after each conditioning phase (HAB, ACQ1, ACQ2, EXT). SCRs were assessed during conditioning. Understanding the underlying brain mechanisms in different back pain stages might help to identify risk and resilience factors that drive the transition from acute to chronic back pain. In the following sections I will discuss the fMRI data acquired during appetitive (section 3.1) and aversive conditioning (section 3.2) during both acquisitions (ACQ1 and ACQ2). I hypothesize that patients with CBP will show impaired acquisition of appetitive learning with a shift away from reward-related brain areas and heightened learning responses during aversive learning with a shift towards emotion-related brain areas, when compared to HC. I propose that already the processing of affective sensory stimuli is impaired in patients with SABP and CBP, leading to maladaptation in learning mechanisms. Lastly, I hypothesize that SABP patients are a heterogeneous group representing a continuum between HC and CBP patients, reflecting brain activation maps with some shared similarities with both groups. Hypothesis based findings are highlighted (section starting with the corresponding hypothesis). Data of the within group effects during conditioning are included in the results section, since learning-related group comparison are based on these data and they are necessary to clarify findings in the group contrasts, but they were not the main focus of this thesis. Therefore, these data are shown, but will not be further discussed. Corresponding behavioural analyses of the subjective ratings and the SCRs can be found in the suppl. (section 6).

3.1 Appetitive conditioning data

The investigation of the appetitive conditioning data revealed that both pain patient groups showed differential evaluation of the appetitive US and the appetitive CS+, on behavioural level. CBP patients showed the lowest pleasantness ratings, whereas the SABP patients showed the highest pleasantness ratings (suppl. Fig. 26). Furthermore, patients with CBP showed higher arousal levels than HC and SABP patients (suppl. Fig. 25). All subject groups learned the coupling between CS+ and US during both acquisitions (suppl. Fig. 24). Brain activity maps elicited by CS+ un. and CS- trials during conditioning were not significantly different within subject groups (Figures 4-9), for that reason the appetitive learning contrast within each group failed to elicit significant BOLD responses (CS+ un. > CS-). Processing during appetitive sensory stimulation (US trials) showed divergent brain activity maps within subject samples, with HC showing significant activation in the somatosensory and OFC, patients with SABP in the precuneus cortex (PC) and patients with CBP in the IC and

somatosensory cortex. Group comparisons revealed that HC showed a stronger activation in the Hipp and the Amy during appetitive learning and weaker BOLD responses in the parietal lobe (PL) when compared to patients with SABP. In contrast to patients with CBP, HC showed a stronger activation in the Hipp and the NAC and weaker activation in the posterior cingulate cortex (pCC). Correlational analyses indicated that initial responses during an appetitive sensory stimulation in the HAB affected the learning responses in all subject samples, with differential involvement of brain areas (Tab. 12). Both pain patient groups revealed an association between initial US brain responses and appetitive learning in the mPFC, whereas HC and patients with SABP both indicated a relationship in the OFC, but with contrary interrelations.

3.1.1 Whole-brain analyses of appetitive learning-related brain activation within subject groups during ACQ1

Investigation of the mechanisms underlying ongoing event-related activation in the brain can help elucidate which activation patterns are really distinct from each other and help to identify maladaptive differences between groups. Underlying brain circuits involved in the processing of the in the experiment used stimuli were characterised in both conditioning paradigms, as well as during appetitive and aversive learning (CS+ un. > CS-) within each subject sample. In the following section the findings of the appetitive learning experiment will be discussed. Aversive conditioning data can be found in section 3.2. Whole-brain analyses of the fMRI data during appetitive conditioning revealed that CS+ un. and CS- trials elicited similar BOLD responses within each group, respectively, therefore the appetitive learning contrasts did not yield significant results. Brain activation maps in CBP patients showed the greatest difference between CS+ un. and CS- trials in ACQ1 and HC in ACQ2. US processing within each group showed divergent involvement of different brain areas during appetitive sensory processing across the subject groups, with a significant increase in BOLD response in the somatosensory cortex in HC, in the PC in SABP patients and in both IC and somatosensory cortex in CBP patients.

HC showed significant BOLD responses for all three stimuli (Fig. 4 and Tab. 3) presented during ACQ1, but not for the appetitive learning contrast between CS+ un. trials > CS- trials. BOLD activation patterns during CS+ un. and CS- processing were comparable, activating both, frontal and lateral regions of the brain, as well as the IC and Tha (Fig. 4A-B). The CS+ un. activated five clusters, whereas the CS- activated three clusters (Tab. 3). Processing of the US activated two clusters in the postcentral gyrus (poG), one cluster in each hemisphere (Fig. 4C and Tab. 3).

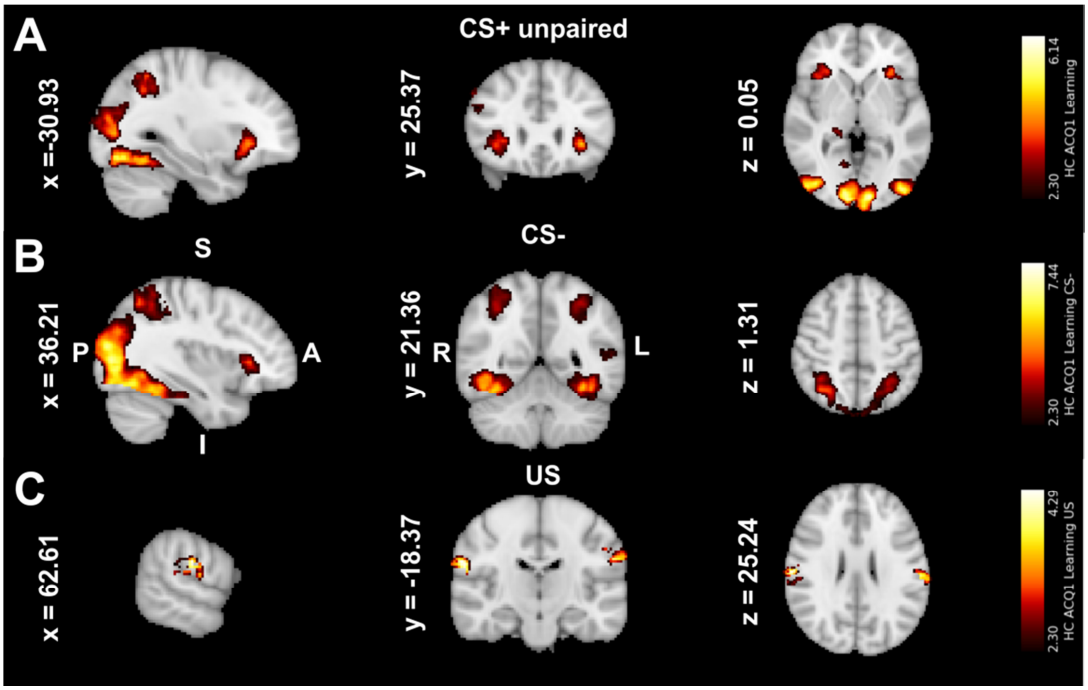

Figure 4: Trial-averaged blood oxygenation level dependent (BOLD) response during appetitive conditioning in HC in ACQ1 (A) during CS+ un., (B) CS- and (C) US processing, respectively. fMRI whole-brain data of HC, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in Montreal Neurological Institute (MNI) space, corresponding to anterior-posterior (A = anterior, P = posterior), inferior-superior (I = inferior, S = superior) and left-right axes (L = left, R= right). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 5 clusters involving the insular cortex (IC), the frontal orbital cortex, the middle frontal gyrus (mFG), the angular gyrus (AG), thalamus (Tha), as well as the occipital lobe (OL). **B:** CS- trials activated similar brain regions as during CS+ un. trials, activating 3 significant clusters in occipital, frontal and inferior regions. **C:** US stimuli activated two clusters in the postcentral gyrus (poG), one in each hemisphere. The learning contrast between CS+ un. trials > CS- trials did not yield any significant results. Cluster details can be seen Tab. 3.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	13176	6.14	3.67	-76.20	5.48	R	67% Lateral Occipital Cortex 56% Thalamus
	422	4.40	36.70	24.60	-0.73	R	41% Insular Cortex 30% Frontal Orbital Cortex
	390	3.67	56.00	-42.90	35.30	R	35% Angular Gyrus 30% Supramarginal Gyrus 87% Inferior parietal lobule PFm
	369	4.68	-32.40	22.50	-4.16	L	62% Insular Cortex 48% Frontal Orbital Cortex
	319	3.43	48.60	17.70	36.0	R	40% Middle Frontal Gyrus
CS-	23454	7.44	0.36	-75.5	6.25	R	39% Frontal Operculum Cortex 50% Visual cortex V4 38% Visual cortex V3
	378	3.9	-32.3	23.9	0.55	L	52% Insular Cortex
	304	4.37	34.8	25.9	2.9	R	37% Frontal Operculum Cortex 34% Insular Cortex
US	272	3.9	-62.4	-21.9	29.4	L	53% Postcentral Gyrus 37% Supramarginal Gyrus anterior 31% Primary somatosensory cortex
	355	4.29	61.2	-19.5	22.1	R	50% Postcentral Gyrus 48% Secondary somatosensory cortex

Table 3: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in HC. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the highest number of clusters, whereas the CS- processing activated the largest cluster. US activation could be found in both hemispheres in the poG. There were no significant results for the appetitive learning contrast between CS+ un. > CS-. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R = right, L = left).

SABP patients showed significant brain activation patterns for the CS+ un., the CS- and the US processing (Fig. 5 and Tab. 4) during ACQ1, but not for the appetitive learning contrast (CS+ un. > CS-). Both CS+ un. and CS- showed peak activations in similar parts of the brain, as the right IC and the precentral gyrus (prG) (Fig. 5A-B). US BOLD response was less spread throughout the brain, involving mainly the PC during PT stimulation (Fig. 5C).

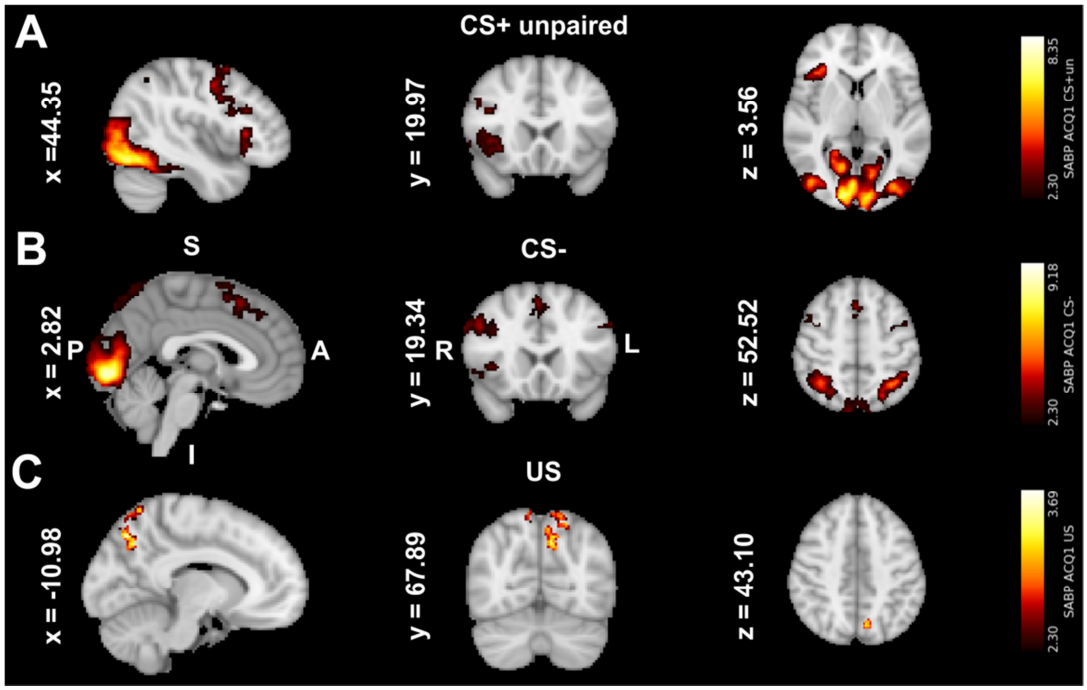

Figure 5: Trial-averaged BOLD responses during appetitive conditioning in SABP patients in ACQ1 (A) during CS+ un., (B) CS- and (C) US processing, respectively. fMRI whole-brain data of SABP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in Montreal Neurological Institute (MNI) space, corresponding to anterior-posterior (A = anterior, P = posterior), inferior-superior (I = inferior, S = superior) and left-right axes (L = left, R= right). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars depict Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 5 clusters involving the IC, the lingual and precentral gyri (prG) and the superior parietal lobe (PL). **B:** CS- trials activated 5 significant clusters in occipital, frontal regions and the prG bilateral, as well as the IC. **C:** US stimulation showed significant BOLD activation in a cluster covering the PL and the prG. There were no significant clusters for the appetitive learning contrast. Cluster details can be seen in Tab. 4.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	17721	8.35	4.8	-76	2.98	R	41% Lingual Gyrus, 84% Visual cortex V1 BA17
	680	4	42.5	7.64	39.8	R	45% Precentral Gyrus
	611	6.34	37.7	25.1	-0.40	R	44% Insular Cortex 40% Frontal Orbital Cortex
	508	4.57	-30.9	-55.4	47.1	L	42% Superior Parietal Lobule 30% Anterior intra-parietal sulcus hIP3
	495	4.76	60.3	-41	20.8	R	42% Superior Parietal Lobule
CS-	26139	9.18	2.55	-74.5	4.77	R	53% Supramarginal Gyrus
	1156	5.14	45.4	15.4	36.7	L	53% Precentral Gyrus
	523	3.89	3.27	16.3	54.5	R	46% Superior Frontal Gyrus 30% Premotor cortex BA6
	480	4.87	-44.3	8.79	38.6	L	31% Precentral Gyrus
	399	4.96	37.2	26	1.04	R	41% Insular Cortex 28% Frontal Operculum Cortex
US	584	3.69	-6.1	-65.8	57.4	L	49% Precuneus Cortex

Table 4: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in SABP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. Both CS+ un. and CS- showed a significant increase in the BOLD responses in 5 clusters covering similar brain region, such as the prG and the IC. US stimulation activated a cluster in the PL and the precuneus cortex (PC). There was no significant BOLD response in the learning contrast. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

CBP patients showed a significant BOLD increase for the processing of the CS+ un. and CS-, but not for the appetitive US or the learning contrast during ACQ1 (Fig. 6 and Tab. 5). CS+ un. activation maps showed an increase in the visual cortex (VC) and the right parahippocampal cortex (Fig. 6A). BOLD responses during CS- processing involved more brain areas, activating three significant clusters within the right hemisphere of the brain (Fig. 6B). The CS- trials activated one large cluster stretching through the right hemisphere, with increased BOLD responses in hippocampal and parietal parts of the brain beside the occipital lobe (OL). Two more clusters could be seen in the IC and the prM. Both, CS+ un. and CS- trials, showed significant increases in the right hemisphere (Tab. 5). The processing of the appetitive US did not result in a significant increase of BOLD. Non-cluster corrected US trials showed a small cluster in the poG (Fig. 6C and Tab. 5).

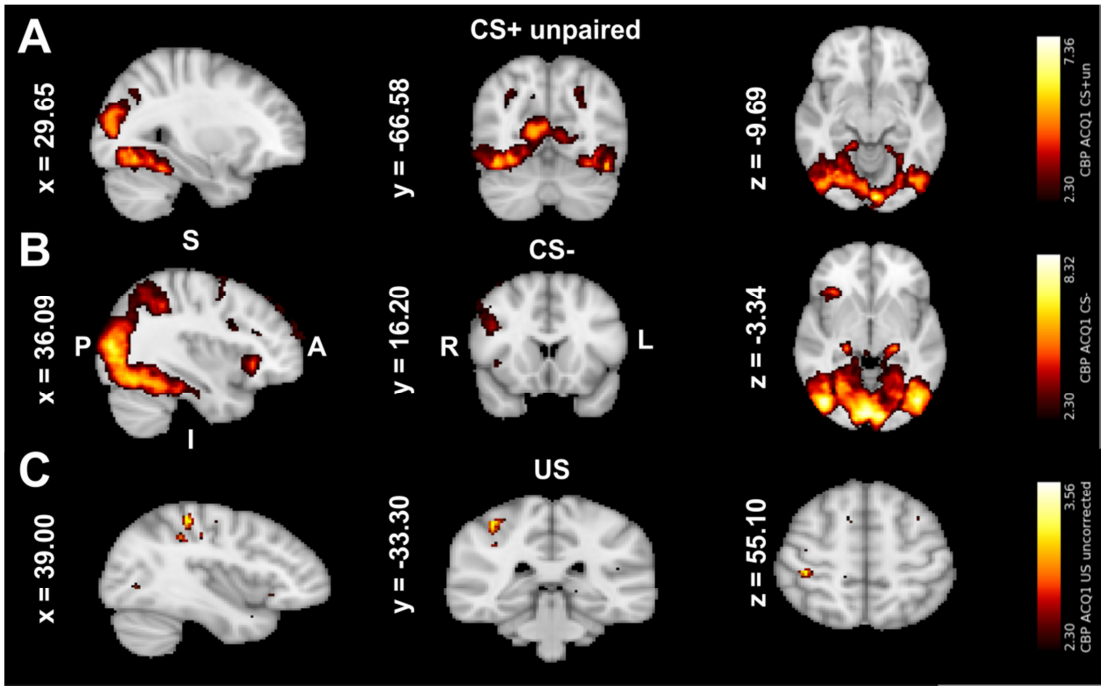

Figure 6: Trial-averaged BOLD responses during appetitive conditioning in CBP patients in ACQ1 (A) during CS+ un., (B) CS- and (C) US processing, respectively. fMRI whole-brain data of CBP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in Montreal Neurological Institute (MNI) space, corresponding to anterior-posterior (A = anterior, P = posterior), inferior-superior (I = inferior, S = superior) and left-right axes (L = left, R= right). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars depict Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 1 large cluster in the lingual gyrus (LG) stretching to the posterior part of the parahippocampal gyrus. **B:** CS- trials activated 3 significant clusters in occipital and frontal areas of the as well as the IC. The cluster in the OL stretched to the hippocampal area and to the PL. Both CS+ un. and CS- showed stronger involvement of the right hemisphere. **C:** US stimuli did not show any clusters in the corrected data set. Uncorrected data (not cluster corrected) revealed at a Z-level = 2.3, a small cluster (74 Voxel) in the poG. Cluster details can be seen in Tab. 5.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	13956	7.36	1.86	-77.2	2.48	R	51% Visual cortex 45% Lingual Gyrus 41% Parahippocampal Gyrus posterior division 72% Hippocampus subiculum
CS-	27807	8.32	2.52	-72.9	4.42	R	58% Lateral Occipital Cortex 62% Hippocampus cornu ammonis
	1558	5.14	43.6	21.8	37.3	R	35% Middle Frontal Gyrus 39% Premotor cortex
	365	3.89	38.6	24.1	-2.41	R	37% Insular Cortex
US	74	3.56	39.0	-33.3	55.1	R	50% Postcentral Gyrus

Table 5: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in CBP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. CS+ unpaired BOLD responses were found in the LG. Significant clusters could be seen in the hippocampus (Hipp), IC and prM for the CS- trials. The cluster given for the US stimulus was not cluster corrected ($p < .05$, $Z = 2.3$). There was no significant cluster in the learning contrast during ACQ1. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

3.1.2 Whole-brain analyses of appetitive learning-related brain activation within subject groups during ACQ2

During appetitive conditioning HC elicited significant BOLD responses for all three stimuli (Fig. 7 and Tab. 6) presented during ACQ2, but not for the appetitive learning contrast. Activation during CS+ un. trials showed a significant increase in the BOLD response mainly in the OL, including the lingual gyrus (LG) (Fig. 7A). While the CS- activated frontal and posterior regions of the brain, as well as subcortical structures such as the Tha and the pCC (Fig. 7B). Processing of the US activated a cluster in the frontal medial cortex (fMC) (Fig. 7C and Tab. 6).

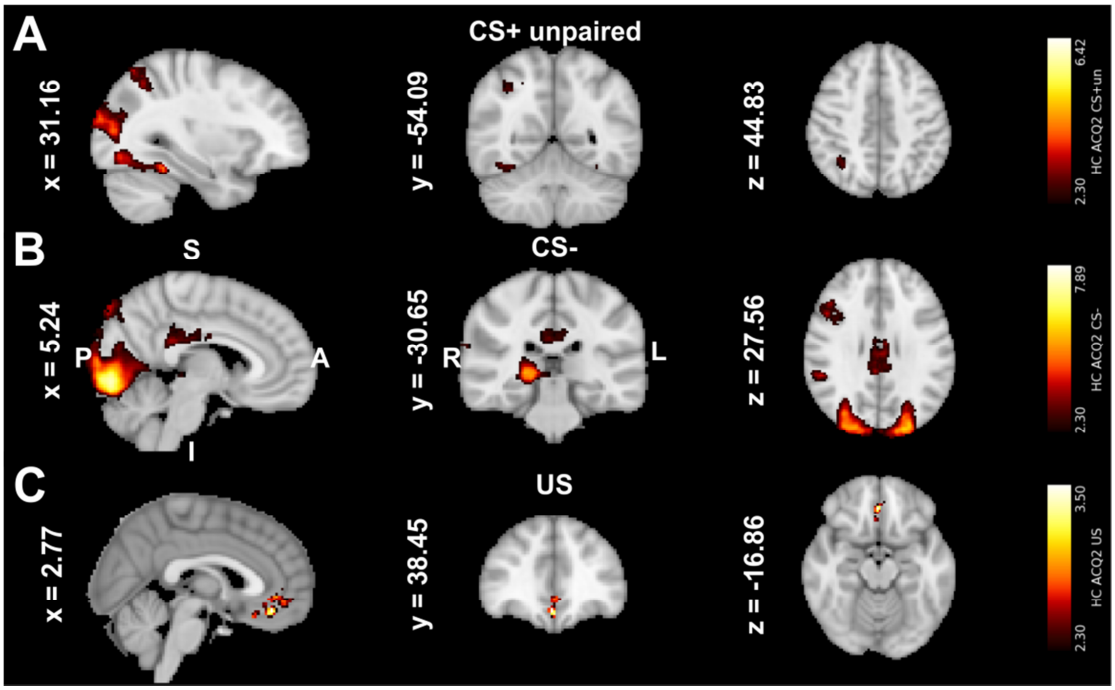

Figure 7: Trial-averaged BOLD responses during appetitive conditioning in HC in ACQ2 (A) during CS+ un., (B) CS- (B) and (C) US processing, respectively. fMRI whole-brain data of HC, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 2 clusters involving frontal and occipital portions of the brain. **B:** CS- trials activated 5 significant clusters covering cortical and subcortical regions, such as the Tha and the posterior cingulate cortex (pCC). **C:** US stimulation resulted in an increased BOLD response in the frontal medial cortex (fMC). There were no significant clusters for the appetitive learning contrast. Cluster details can be seen in Tab. 6.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	7381	6.14	2.51	-84.3	2.17	R	59% Lingual Gyrus
	307	3.76	33.3	-60.8	51.9	R	58% Lateral Occipital Cortex
CS-	22766	7.89	1.63	-77.3	5.88	R	61% Lingual Gyrus
	598	4.27	0.74	-29	27.7	R	86% Cingulate Gyrus posterior division
	582	3.94	45.7	12.6	30.3	R	37% Inferior Frontal Gyrus
	388	5.46	18.8	-30.6	-0.37	R	93% Right Thalamus
	348	4.15	56.8	-40.3	27.8	R	46% Supramarginal Gyrus
US	252	3.5	1.73	41.2	-8.98	R	76% Frontal Medial Cortex

Table 6: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in HC. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain

brain area and its probability. The CS+ un. involved mainly the visual cortex (VC), whereas the CS- processing activated 5 clusters, the largest cluster covering both cortical and subcortical brain areas, such as the Tha and the pCC. Processing of the appetitive US showed an increase in BOLD responses in the fMC. Appetitive learning did not yield any significant clusters. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

SABP patients showed significant brain activations for the processing of the CS+ un. and the CS-, but neither for the US nor the appetitive learning contrast (Fig. 8 and Tab. 7) during ACQ2. In contrast to that in ACQ1 also the US processing elicited a significant BOLD response in the PC (e.g. Fig. 5 and Fig. 8). CS+ un. trials and CS- trials showed comparable BOLD responses, involving both IC and the pCC (Fig. 8). CS+ un. trials showed an increase in BOLD responses bilaterally in the IC, whereas CS- showed significant responses only in the right IC (Fig. 8).

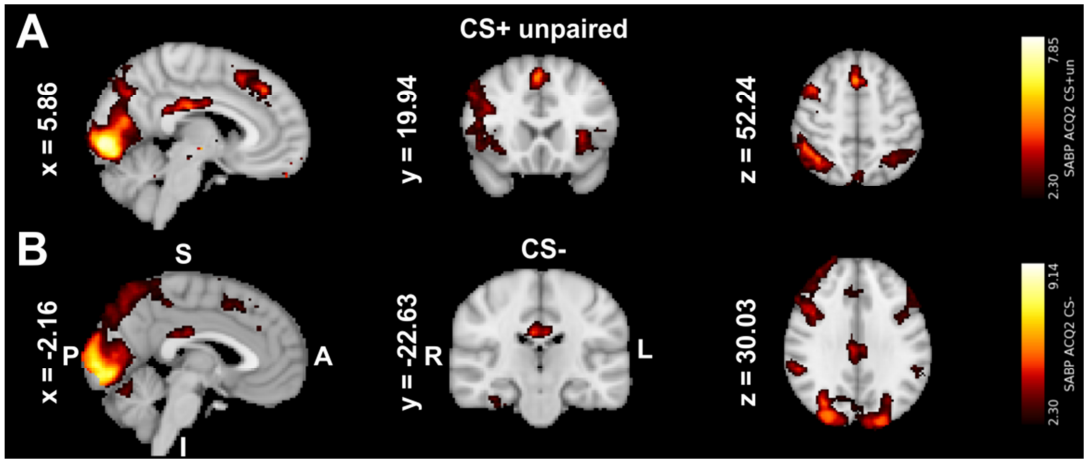

Figure 8: Trial-averaged BOLD responses during appetitive conditioning in SABP patients in ACQ2 (A) during CS+ un., (B) CS- and (C) US processing, respectively. fMRI whole-brain data of SABP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials showed significant BOLD activation in 5 clusters, covering the frontal cortex, IC, the cingulate and prG. **B:** CS- trials activated 4 significant clusters in occipital and frontal areas. Neither US stimulation nor the appetitive learning contrast did not show any clusters in the cluster corrected data set. Cluster details can be seen in Tab. 7.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	20434	7.85	8.46	-72.6	9.20	R	65% Lingual Gyrus
	3419	5.56	40.3	27.8	22.5	R	65% Precentral Gyrus 61% Insular Cortex
	653	5.31	2.59	26.2	46.9	R	54% Paracingulate Gyrus
	539	5.49	1.82	-25.3	27.6	R	71% Cingulate Gyrus posterior division
	408	4.45	-34.1	23.2	0.80	L	53% Insular Cortex
CS-	33947	9.14	4.11	-71.6	9.59	R	63% Lingual Gyrus
	5699	5.21	32.3	27.8	31.2	R	36% Frontal Orbital Cortex 61% Insular Cortex
	749	3.71	-46.8	14.2	34.8	L	44% Middle Frontal Gyrus
	456	5.02	1.58	-26.7	27.7	R	72% Cingulate Gyrus posterior division

Table 7: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in SABP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. Only CS+ un. and CS- trials showed a significant increase in the BOLD response, activating similar brain regions. CS+ un. processing involved the cingulate gyrus (CG) and prG, as well as the IC. The CS- BOLD response also involved the CG and the IC, but only in the right hemisphere, besides the LG and parts of the frontal cortex. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

CBP patients elicited significant BOLD responses for all presented stimuli, during appetitive conditioning (Fig. 9 and Tab. 8), but not for the appetitive learning contrast. Processing of the CS+ un. trials involved more brain areas in the right hemisphere (Fig. 9A), whereas the CS- and the US stimuli also showed activations in the left hemisphere (Fig. 9B). CS+ un. and CS- trials showed similar BOLD responses in the prM the prG, Tha and the IC. In contrast to ACQ1, also the US trials showed significant brain activation during ACQ2 in the IC, the prG and the S2 (e.g. Fig. 6C and Fig. 9C).

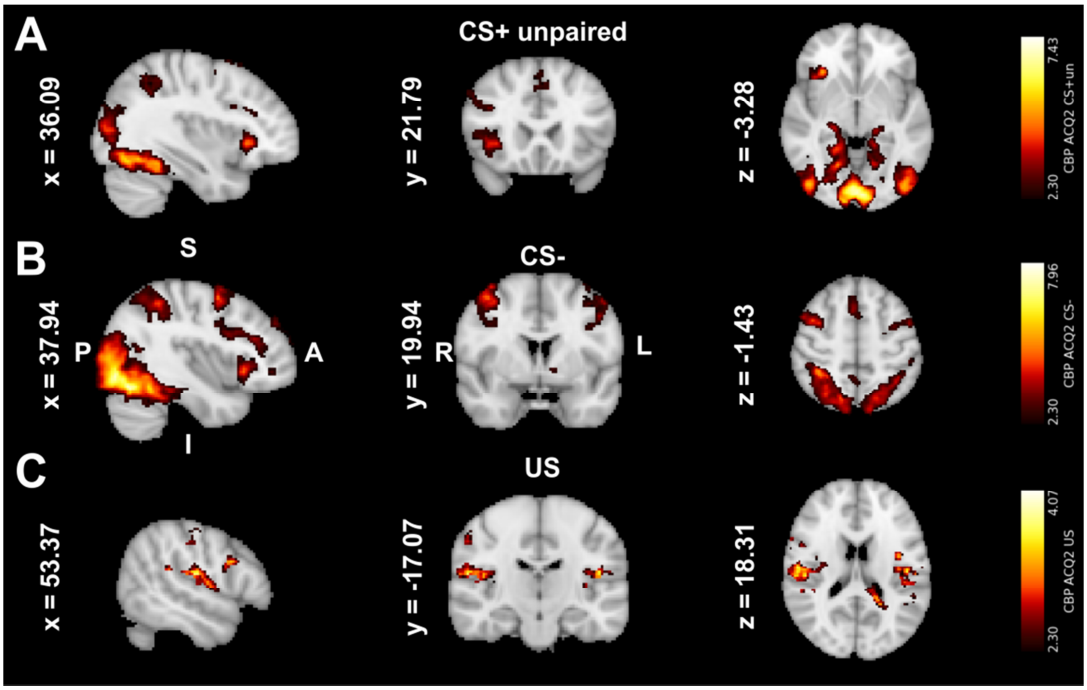

Figure 9 Trial-averaged BOLD responses during appetitive conditioning in CBP patients in ACQ2 (A) during CS+ un., (B) CS- and (C) US processing, respectively. fMRI whole-brain data of CBP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 4 clusters in the anterior cingulate cortex (ACC), IC, prM and prG, in the right hemisphere. **B:** CS- stimuli showed a significant BOLD response in 6 clusters in similar brain regions as during CS+ un- processing, including the IC and prG, prM, paracingulate, Tha and occipital parts of the brain. In addition, there was also an increase in activation in the left hemisphere of the Hipp, prG and the pCC. **C:** US stimulation elicited significant brain activation in the opercular cortex, the prG and the IC. There were no significant clusters in the appetitive learning contrast. Cluster details can be seen in Tab. 8.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
CS+ un.	17007	7.43	3..4	-74.2	0.79	R	51% Occipital Pole 49% Lingual Gyrus 38% Visual cortex V1 BA17 73% Hippocampus subiculum
	729	3.71	44.2	14.0	41.4	R	55% Middle Frontal Gyrus Cortex 30% Precentral Gyrus
	534	3.90	1.13	20.2	47.8	R	47% Paracingulate Gyrus 61% Premotor
	388	5.20	37.6	24.8	-0.57	R	42% Insular Cortex 27% Frontal Orbital Cortex
CS-	34234	7.96	2.5	-71.2	6.74	R	67% Thalamus 45% Lingual Gyrus 44% Lateral Occipital Cortex 44% Parahippocampal 43% Visual cortex V1 BA17 36% Hippocampus
	2085	4.91	42.3	16.8	26.1	R	40% Insular Cortex 33% Precentral Gyrus 30% Middle Frontal Gyrus
	559	3.75	2.05	17.6	49.1	R	56% Paracingulate Gyrus 37% Premotor cortex
	484	3.69	-41.9	2.64	43	L	45% Precentral Gyrus 35% Premotor Cortex
	479	3.39	35	53.8	26.7	R	85% Frontal Pole
	373	3.52	-0.67	-29.5	25.8	L	64% Cingulate Gyrus posterior division
US	899	4.07	-37.8	-28.7	17.2	L	45% Insular Cortex 32% Central Opercular Cortex
	698	3.71	51.1	-14.5	17.8	R	54% Central Opercular Cortex 50% Secondary somatosensory cortex
	287	3.92	56.6	9.97	23.2	R	56% Precentral Gyrus 40% Broca's area BA44

Table 8: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS- and US processing in CBP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. BOLD responses for the CS+ un. could be seen only in the right hemisphere, whereas both CS- and US showed significant increases in both hemispheres. CS+ un. and CS- trials showed a significant increase in BOLD responses in similar brain regions, including the prM, prG and IC. Appetitive learning did not yield any significant clusters. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

Hypothesis 1: Impaired acquisition of appetitive learning in CBP

3.1.3 Group comparison of appetitive learning-related brain activation in ACQ1

In this section focus will be laid on learning-related increases in BOLD responses between both pain patient groups and both in contrast to HC. Therefore, brain activation patterns between two subject groups were always contrasted and statistically compared. Firstly, in the whole-brain data, and secondly, in the ROI data.

Whole-brain analyses of learning-related brain activation resulted in significant findings between HC and CBP patients during ACQ1 (Fig. 10). HC showed a stronger activation in the Hipp in contrast to CBP patients (Fig. 10 and Tab. 9). A significant increase in BOLD responses could be seen in the ROI analyses in the Hipp (Fig. 10B) and in the NAC (Fig. 10C) in HC when compared to patients with CBP during the acquisition of the appetitive CS+. Both ROIs were activated bilaterally. There were no significant differences in learning-related brain responses in the group comparison between HC and SABP patients, neither in the contrast between CBP and SABP patients.

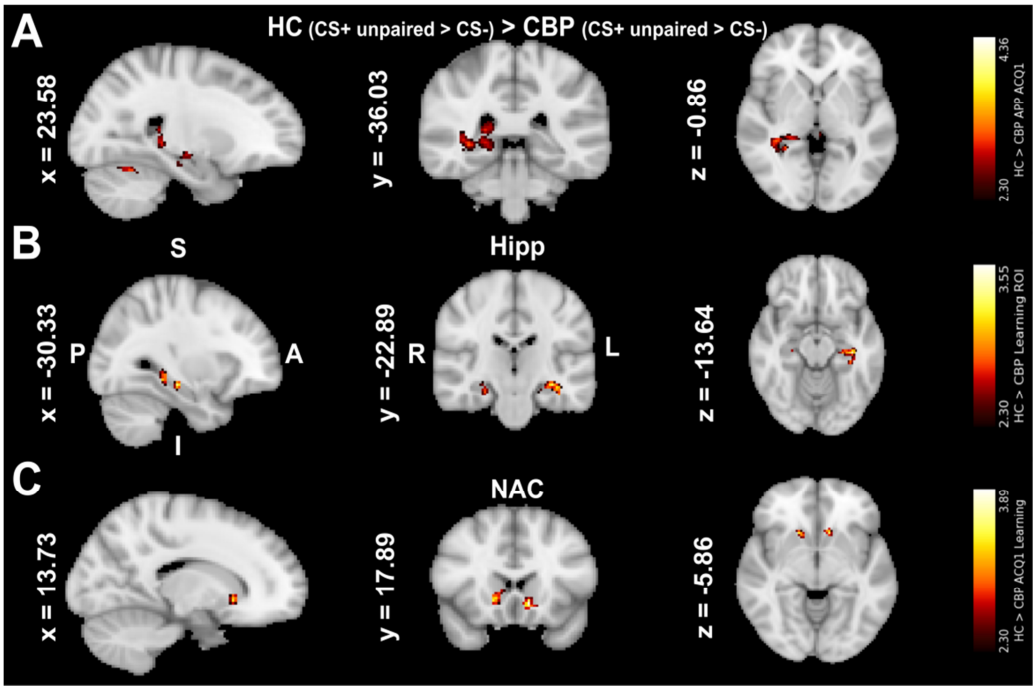

Figure 10: Group comparison of brain activity maps for appetitive learning (contrast CS+ un. > CS-) during ACQ1. Group contrast between HC > CBP (A) whole-brain data, (B) region of interest (ROI) results in the Hipp and (C) in the nucleus accumbens (NAC). Brain masks used in the ROI analyses were selected from the Harvard-Oxford Cortical Structural Atlas. Used masks were covering both sites of the respective brain area. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and

lower activations in red. **A:** HC showed significant learning-related brain activation in the right Hipp, the cingulate cortex and the cerebellum in contrast to CBP patients, in the whole-brain analyses. **B-C:** ROI analyses showed an increase in BOLD responses in the Hipp (B) and the NAC (C) during the acquisition of the appetitive CS+ un. in HC in contrast to CBP patients. Cluster details can be seen in Tab. 9.

CS+ un. > CS-	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
HC > CBP (whole-brain)	492	3.55	23.7	-34.8	1.36	R	48% Right Hippocampus 90% Hippocampus dentate gyrus
	492	3.26	-11	-57.9	34.1	L	39% Precuneus Cortex 30 % Cingulum
	387	4.36	5.72	-58.7	-23.8	R	98% Cerebellum
HC > CBP (Hipp)	213	3.55	-30.3	-30.2	-8.60	L	93% Hippocampus 80% Hippocampus cornu ammonis 34% Hippocampus dentate gyrus
	202	3.21	26.3	-31.7	-3.00	R	40% Hippocampus 32% Thalamus 80% Hippocampus dentate gyrus 67% Hippocampus cornu ammonis
HC > CBP (NAC)	67	3.4	12.5	16.1	-3.02	R	36% Accumbens 30% Caudate
	51	3.89	-10.1	17.9	-7.89	L	36% Accumbens

Table 9: Cluster list of increased BOLD responses during appetitive learning in the group comparison of HC > CBP patients. All depicted values were estimated for the location of the peak voxel within the given cluster in the upper panel for the whole-brain data and in the two lower panels for the ROI analyses. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The Hipp showed an increase in BOLD responses in both whole-brain data and in the ROI data in HC when compared to CBP patients. Additionally, the NAC showed also stronger activation in HC in comparison to CBP patients. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

3.1.4 Group comparison of appetitive learning-related brain activation in ACQ2

In contrast to ACQ1 both, SABP and CBP patients showed stronger learning responses when contrasted to HC during ACQ2. SABP patients showed significant activation of the parietal operculum (PO) in contrast to the HC sample (Fig. 11A and Tab. 10), whereas CBP patients showed stronger learning-related brain activation in the Hipp and pCC (Fig. 11B and Tab. 10), than the HC sample. Still there was no significant difference between patients with SABP and CBP.

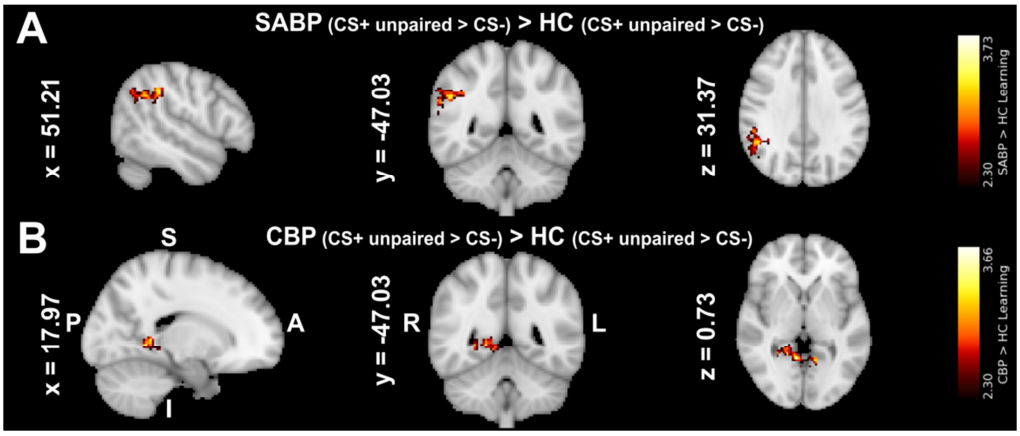

Figure 11: Group comparison of brain activity maps for appetitive learning (contrast CS+ un. > CS-) during ACQ2. BOLD responses (whole-brain) during appetitive learning in the group contrast: (A) SABP > HC, (B) CBP > HC. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** SABP patients showed stronger activation of the right parietal operculum (PO) and parts of the inferior parietal cortex when compared to HC. **B:** CBP patients showed significant learning-related brain activation in the pCC for the acquisition of the appetitive CS+ un. > CS-. Cluster details can be seen in Tab. 10.

In the ROI analyses only the contrast between HC and SABP patients resulted in a significant increase in BOLD responses. HC showed significant activations in the Amy (Fig. 12A) and the Hipp (Fig. 12B) when compared to SABP patients. Cluster details can be found in Tab. 10. There were no significant differences in ROI analyses between HC and CBP patients and in the comparison between both pain patient groups.

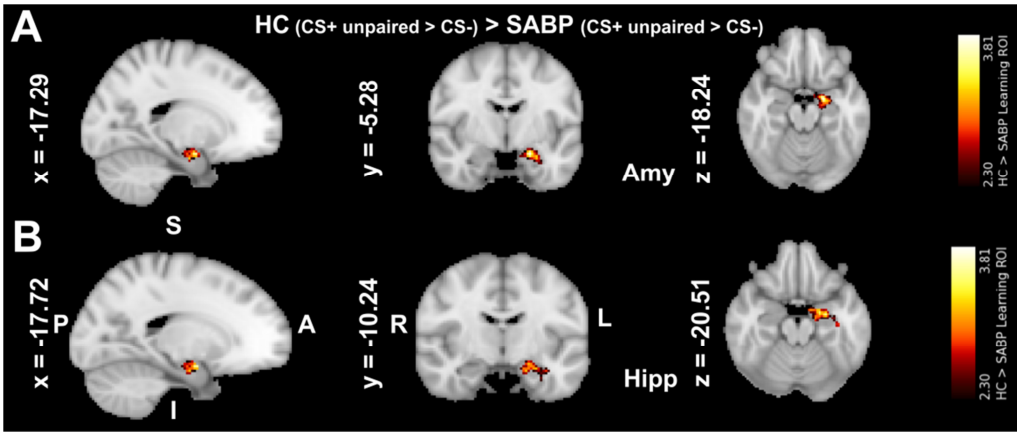

Figure 12: Group comparison of brain activity maps for appetitive learning (contrast CS+ un. > CS-) during ACQ2. ROI analyses in the (A) Hipp and (B) amygdala (Amy) in the group contrast between HC > SABP. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right

axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. Only the left Hipp and left Amy showed a significant increase in BOLD responses during appetitive learning in HC in contrast to SABP patients. Cluster details can be seen in Tab. 10.

CS+ un. > CS-	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	*Hemi.*	*Brain Structure*
SABP > HC (whole-brain)	732	3.73	4.07	-79.2	-2.05	R	40% Lingual Gyrus 80% Visual cortex V1 BA17
	423	3.44	53.09	-48.4	31.2	R	55% Angular Gyrus 48% Inferior parietal lobule PFm 40% Inferior parietal lobule Pga 37% Parietal Operculum Cortex
CBP > HC (whole-brain)	366	3.66	8.95	-51.4	0.03	R	43% Cingulate Gyrus posterior division
HC > SABP (Amy)	215	3.81	-20.9	-7.47	-19.4	L	81% Amygdala 90% Amygdala laterobasal group 38% Amygdala superficial group
HC > SABP (Hipp)	218	3.81	-23.3	-9.54	-20.3	L	74% Left Hippocampus 80% Hippocampus cornu ammonis

Table 10: Cluster list of increased BOLD responses during appetitive learning in the group comparison between pain patients and HC (whole-brain), and in HC in contrast to SABP and CBP patients (ROI). All depicted values were estimated for the location of the peak voxel within a given cluster in the whole-brain data (upper two panels) and for the ROI analyses (lower two panels). Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. Whole brain-analyses revealed an increase in BOLD responses in both pain patient groups (SABP and CBP) when contrasted to HC. In contrast to that, ROI analyses resulted in stronger BOLD responses in HC in the left Amy and left Hipp when contrasted with SABP patients. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

Hypothesis 3: Impaired appetitive sensory processing in back pain patients

3.1.5 Interaction between fMRI data and behavioural responses

The previous section indicated that learning mechanisms in the subject groups seem to be divergent, during appetitive conditioning. To test whether the US evaluation, on a behavioural level as well as the physiological responses elicited by the appetitive sensory stimulation, had an impact on those divergent BOLD responses during learning, the US responses during the HAB were considered in the analyses. All subjects had the first contact with the PT during the HAB (fMRI data not shown). In a first step, the valence ratings of the PT during HAB were correlated with the estimated learning responses in the different ROIs, within each group, respectively. Correlational analyses were only tested within each group separately, to investigate the impact of the PT evaluation and its elicited brain responses on learning-related brain activation. Significant correlation coefficients between US valence ratings and learning responses in the ROI analyses could only be found in patients with SABP. They showed a negative, but significant correlation between US valence ratings and the learning-related brain responses in the Hipp ($r(46) = -.302$, $p = .039$) during ACQ1 (Tab. 11). Furthermore, in ACQ2 significant correlation could be seen in the IC ($r(46) = -.408$, $p = .004$) and the Amy ($r(46) = -.338$, $p = .020$) (Fig. 13 and Tab. 11).

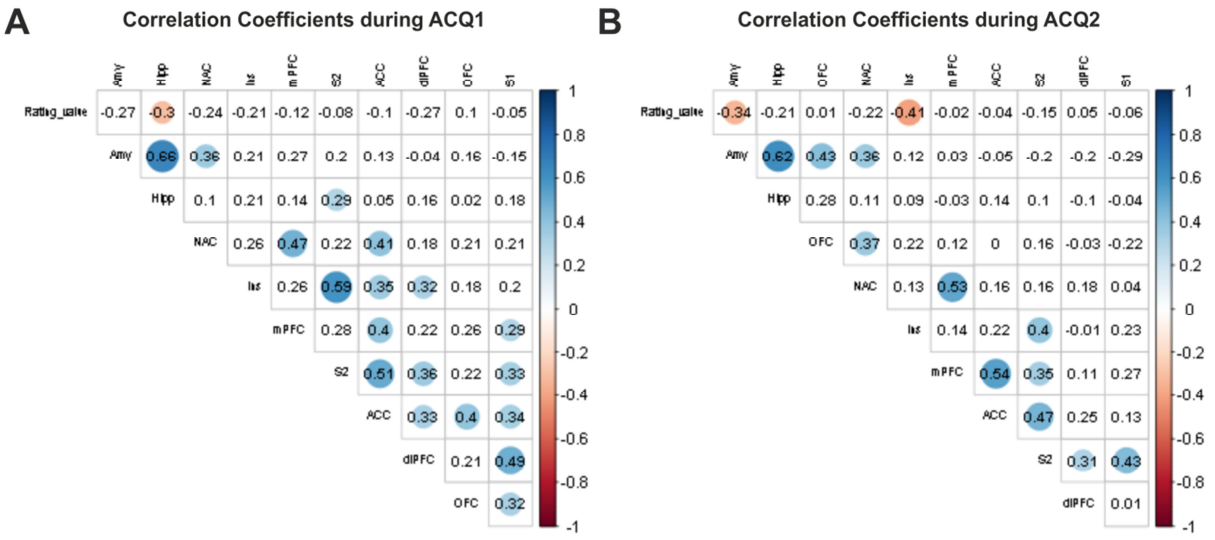

Figure 13: Correlation matrix: US valence ratings and learning-related BOLD responses in SABP patients. Spearman correlation (two-tailed) was calculated between BSC during appetitive learning (CS+ un. > CS-), during (A) ACQ1 and (B) ACQ2 in different ROIs and US valence ratings in the HAB. Spearman correlation was chosen to account for the non-normally distributed data of the US valence rating data in the HAB. Correlation coefficients (Spearman's rho) are depicted in the first row of the correlation matrix (Rating_value). Significant correlations are highlighted with coloured circles, in blue for a positive correlation and in red for a negative correlation. Circle size represents significance thresholds with $p < .05$ (small circle), $p < .01$ (medium circle) and $p < .001$ (large circle) with corresponding significance levels depicted on the colour bar. **A-B:** SABP patients

showed a negative correlation between US valence ratings and appetitive learning responses in both acquisitions. There was a weak negative correlation in the Hipp (ACQ1) and weak to moderate negative correlation in the Amy and in the IC (ACQ2). Numbers = Spearman's rho, circles = significance level, Amy = amygdala, Hipp = hippocampus, NAC = nucleus accumbens, IC = insular cortex, mPFC = medial prefrontal cortex, S2 = secondary somatosensory cortex, ACC = anterior cingulate cortex, dlPFC = dorsolateral prefrontal cortex, = orbitofrontal cortex, S1 = primary somatosensory cortex. Correlation coefficients are also depicted in Tab. 11.

Correlation Coefficients

group	*Phase*	*ROI*	*statistics*		*df*
SABP	ACQ1	Hipp	r	-0.302 *	46
			p	0.039	
SABP	ACQ2	IC	r	-0.408 **	46
			p	0.004	
		Amy	r	-0.338 *	
			p	0.020	

Table 11: Correlation coefficients: US valence ratings and learning-related BOLD responses during appetitive learning. Spearman correlation coefficients were calculated between learning-related brain activation in different ROIs, during appetitive conditioning (ACQ1 and ACQ2) and US valence ratings in the HAB. SABP patients showed a significant but negative correlation between US valence ratings and learning responses in both acquisitions. Neither HC nor patients with CBP showed significant correlation between US valence ratings and appetitive learning BOLD responses. r = Spearman's rho, p = significance level, df = degrees of freedom, Asterisks depict significant correlations (*p < .05, **p < .01). Hipp = hippocampus, IC = insular cortex, Amy = amygdala.

3.1.6 Correlation between fMRI data during pleasant touch stimulation and appetitive learning responses

In a second step, a linear regression model was used to calculate the association between the BOLD responses during PT stimulation in the HAB and learning-related responses in the ROI data, to test whether the physiological response during an appetitive sensory stimulation can predict the associated learning responses in the subject groups. The linear regression model revealed a significant relationship between brain activation during PT stimulation and learning-related brain activation in all subject groups, showing divergent associations between brain areas. A significant association between ROI activation during PT stimulation and learning-related brain activation was found in SABP patients during ACQ1 and ACQ2. Activation in the mPFC ($F(1,46) = 8.127$, $p = .007$) during PT stimulation explained 15 % of the variance in the learning-related brain responses during ACQ1 and 8.6 % in the OFC ($F(1,46) = 4.25$, $p = .045$). There was also a significant relationship between learning and appetitive sensory processing during ACQ2 in the IC ($F(1,46) = 5.288$, $p = .026$) and the dlPFC ($F(1,46) = 6.827$, $p = .012$) (Tab. 12). In a similar manner, HC showed a significant but negative relationship between US BOLD responses and learning responses in the OFC

F(1,36) = 5.01, p = .032), only during ACQ2. In CBP patients a significant positive relationship between processing of an affective sensory stimulus and learning-related-brain activation was seen in the mPFC (F(1,33) = 4.393, p = .044) during ACQ2 (Tab. 12).

Linear Regression								
group	***Phase***	***ROI***	***Unstandardized Coefficients***		***t***	***R^2***	***Adj. R^2***	***Sig.***
			B	***SE***				
SABP	ACQ1	mPFC	-0.399	0.140	-2.851	0.153	0.134	0.007
		OFC	0.205	0.100	2.062	0.086	0.066	0.045
HC	ACQ2	OFC	-0.324	0.145	-2.238	0.128	0.103	0.032
SABP	ACQ2	dlPFC	0.323	0.124	2.613	0.132	0.112	0.012
		IC	0.251	0.109	2.300	0.105	0.085	0.026
CBP	ACQ2	mPFC	-0.363	0.173	-2.096	0.121	0.093	0.044

Table 12: Linear regression model: processing of an appetitive US during HAB and brain responses during appetitive learning. Dependent variable: learning-related BOLD responses during ACQ1 and ACQ2 in different ROIs. Predictors: Brain responses during appetitive US stimulation during HAB. SABP patients showed a significant relationship between US BOLD responses and the learning-related brain activation in ACQ1 in the mPFC and the OFC and in ACQ2 in the dlPFC and the IC. In contrast to that, HC showed a significant association between PT processing and learning-related responses only in the OFC and CBP patients in the mPFC, during ACQ2. Both pain patient groups exhibited a negative relationship between PT and appetitive learning responses, but temporally separated during ACQ1 in SABP patients and ACQ2 in CBP patients. HC and SABP patients revealed both a dependency between US and learning responses in the OFC, but with contrary relationships (positive in SABP patients, negative in HC). ROI = region of interest, Adj. R^2 = Adjusted R^2, Sig. = Significance level, SE = Standard Error, mPFC = medial prefrontal cortex, OFC = orbitofrontal cortex, dlPFC = dorsolateral prefrontal cortex, IC = insular cortex.

3.2 Aversive conditioning data

Aversive conditioning data did not show any differences between subject samples on a behavioural level (suppl. Figures 29-31). Activity maps during aversive conditioning within the subject groups, respectively, showed divergent brain activity maps for the in the experiment used stimuli, as well as for the aversive learning contrast (Figures 14-19). BOLD responses during CS+ un. trials and aversive learning shared a lot of similarities in brain activations patterns in both pain patient groups during ACQ1, involving the ACC and the prM in both groups. Besides this activation patterns, additional significant increases in BOLD responses which were not shared were found. The shared significant activation maps between CS+ un. and aversive learning included a significant increase of activation in the IC, the putamen and the ACC during ACQ2, only in CBP patients (Fig. 19). HC and SABP patients showed fewer similarities in brain activation patterns between CS+ un. and aversive learning responses, during ACQ2 (Fig. 17 and Fig. 18). Activation maps in the IC showed most prominent group differences during aversive learning, with a divergent involvement of the IC within subject groups (Fig. 21). Furthermore, the aversive conditioning data indicated that the initial brain response during an aversive sensory stimulation in the HAB, affected learning-related brain responses in all subject samples (Tab. 21 and Tab. 22). HC and CBP patients showed an influence of the NAC, during initial aversive painful stimulation in the HAB, on learning responses during ACQ2 (Tab. 22). Besides this relationship there was no comparable association between initial brain responses during the painful stimulation and learning-related brain activation, between subject groups. Patients with SABP revealed that only the OFC responses during the US stimulation, in the HAB, could explain some of the variances in learning responses during ACQ2 (Tab. 22).

3.2.1 Whole-brain analyses of aversive learning-related brain activation within subject groups during ACQ1

HC showed significant BOLD responses for all three stimuli (Fig. 14A-C) presented during ACQ1 of the aversive conditioning paradigm. In addition, the aversive learning contrast between CS+ un. > CS- elicited significant brain responses (Fig. 14D). BOLD activation patterns during CS+ un. and CS- processing were comparable, activating both, the Tha, the pCC, the prG and parts of OL (Fig. 14A-B). Processing of the CS+ un. trials activated the highest number of clusters, whereas the CS- trials activated the largest cluster (Tab. 13). In addition, CS+ un. trials showed significant BOLD responses in the IC and the OFC bilaterally. Processing of the aversive US activated mostly brain areas involved in sensory processes, such as the S2 and the prG. Aversive learning showed significant activations in the right IC and in the lingual cortex (Fig. 14D and Tab. 13).

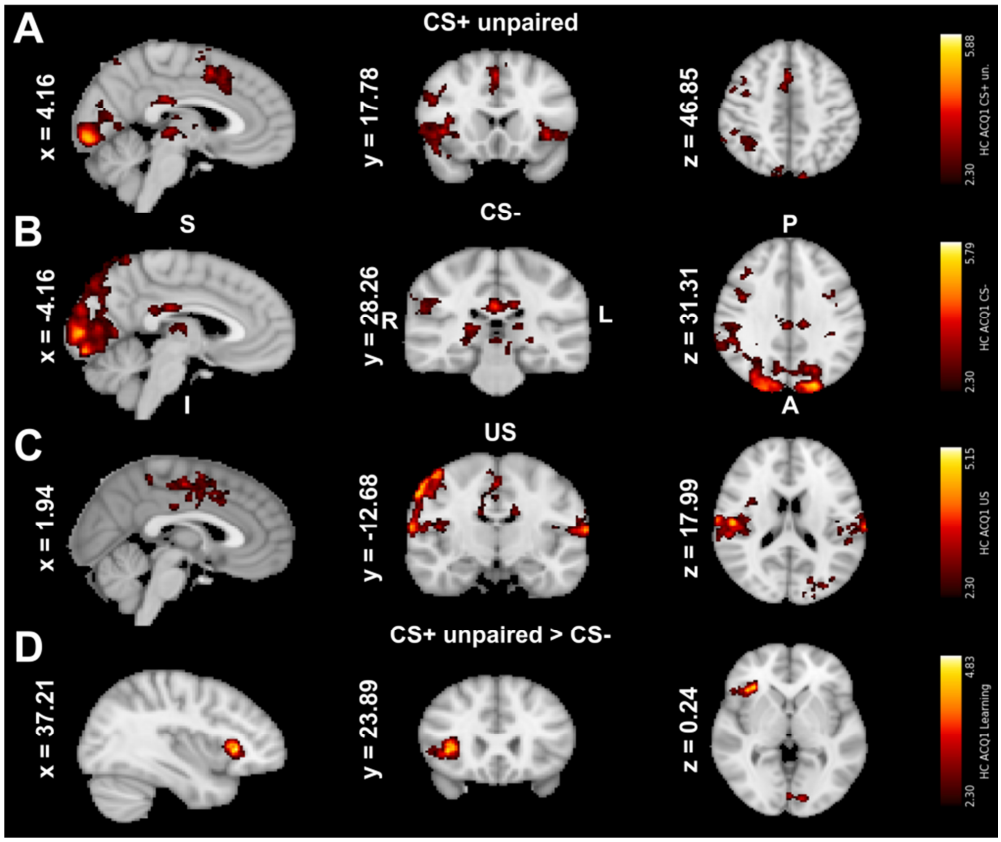

Figure 14: Trial-averaged BOLD response during aversive conditioning in HC in ACQ1 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of HC, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 8 clusters involving the IC bilaterally, the pCC the Tha, as well as the OL. **B:** CS- trials activated similar brain regions as during CS+ un. processing, with 5 significant clusters in the OL, parts of the middle and frontal gyrus, the Tha and the pCC. **C:** US stimuli activated 4 clusters in sensory brain areas, such as the S2 and PO. **D:** The learning contrast between CS+ un. trials > CS- trials activated the right IC. Cluster details can be seen in Tab. 13.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	8925	5.88	0.89	-76.9	3.74	R	80% Temporal Occipital 64% Visual cortex V1 BA17
	1725	3.88	51.4	-43.9	34.8	R	55% Supramarginal Gyrus posterior division 80% Inferior parietal lobule
	1287	5.11	43.1	21.3	-0.80	R	48% Insular Cortex 54% Frontal Operculum Cortex
	877	3.95	6.18	-11.2	-1.05	R	100% Thalamus
	819	3.97	44.7	9.23	37.7	R	47% Precentral Gyrus 30% Broca's Area BA44
	480	4.03	3.84	11	49.9	R	57% Juxtapositional Lobule 70% Premotor Cortex
	476	4.27	-39.6	20	0.09	L	68% Insular Cortex 32% Frontal Operculum Cortex
	402	4.01	0.63	-26.5	27.7	R	40% Cingulate Gyrus posterior division
CS-	18687	5.79	5.95	-72.6	16.2	R	63% Occipital Pole
	972	3.96	5.83	-18.4	4.43	R	100% Thalamus
	504	3.96	42.2	12.8	37.9	R	64% Precentral Gyrus 41% Middle Frontal Gyrus
	473	4.5	-1.11	-27.4	27.6	L	69% Cingulate Gyrus posterior division
	323	3.44	-39.0	6.32	40.5	L	38% Middle Frontal Gyrus 31% Precentral Gyrus
US	4967	5.15	38.1	-17.1	39.7	R	44% Precentral Gyrus
	1282	3.79	-30.1	-81.6	6.94	L	38% Lateral Occipital Cortex, inferior division
	974	4.51	-59.6	-14.2	14.1	L	57% Central Opercular Cortex 38% Secondary somatosensory cortex / Parietal operculum
	621	3.34	35.1	-71.5	-0.90	R	40% Inferior parietal lobule PFop
CS+un. > CS-	483	4.83	38.9	24.3	0.16	R	58% Insular Cortex 51% Frontal Orbital Cortex
	336	4.42	-7.42	-85.5	-5.54	L	57% Lingual Gyrus

Table 13: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in HC. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the highest number of clusters, whereas the CS- processing activated the largest cluster. US activation could be found in mainly in sensory brain areas. Aversive learning contrast between CS+ un. > CS- showed a significant increase in BOLD response in the right IC contralateral to the stimulation site. Stimulus: mean stimulus, Number of Voxels = number of voxels

activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

SABP patients showed significant brain activation patterns for the CS+ un., the CS- and the US processing during ACQ1 and for the aversive learning contrast (Fig. 15 and Tab. 14). CS+ un. trials elicited significant BOLD responses in seven clusters including the IC bilaterally, frontal brain areas such as the ACC, prM and the prG, as well as occipital parts of the brain (Fig. 15A). In contrast to that, the CS- trials only showed significant activation patterns in three clusters in parietal, frontal and occipital parts of the brain, the latter stretching into the Tha and parahippocampal cortex (Fig. 15B). US BOLD responses activated the highest numbers of clusters, with several clusters in the middle and cingulate gyrus (CG), as well as brain areas involved in sensory processing, such as the S2, PO and prG (Fig. 15C). The aversive learning contrast activated three clusters spread through the lingual, paracingulate gyri and the ACC. CS+ un. trials and aversive learning brain activity maps showed a lot of similarities.

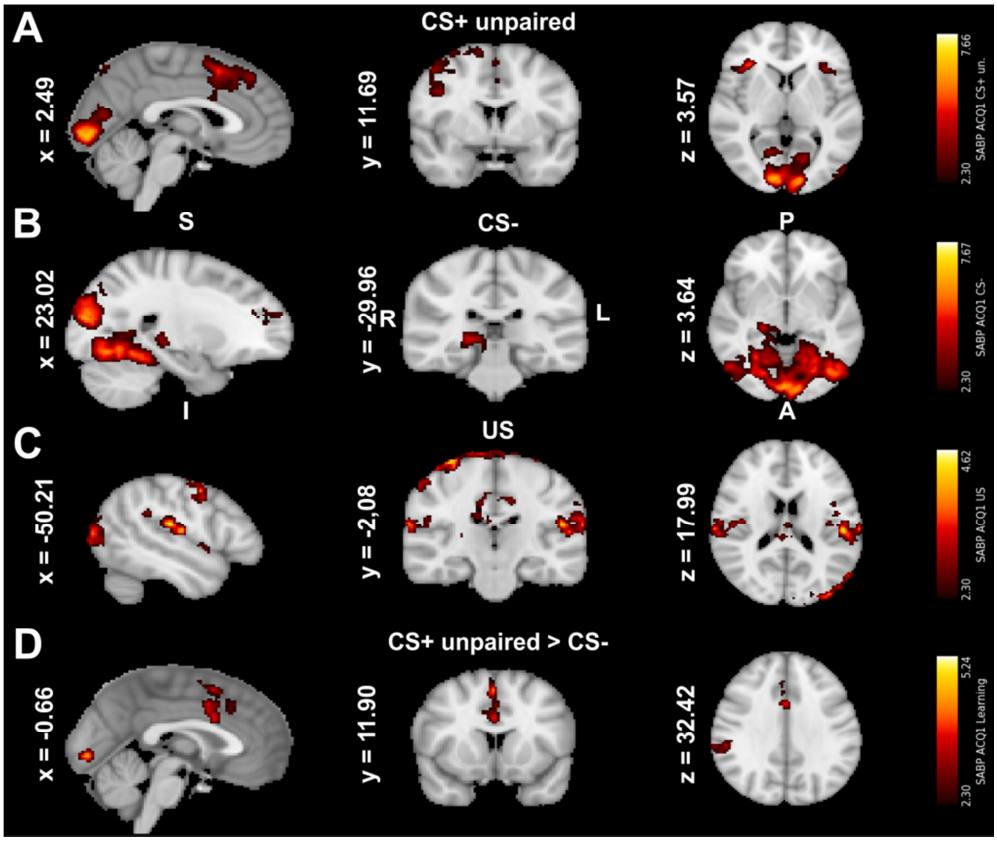

Figure 15: Trial-averaged BOLD response during aversive conditioning in SABP patients in ACQ1 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of SABP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars

represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 7 clusters involving the IC, the LG, ACC, prM and the prG. **B:** CS- trials activated 3 significant clusters in occipital, frontal and parietal regions of the brain, stretching to the parahippocampal gyrus. **C:** US stimulation showed significant BOLD activation in 9 clusters covering the CG, middle gyrus, pCC, PO and the prG. **D:** Aversive learning-related brain activation (CS+ un. > CS-) could be found in frontal brain areas, such as the ACC, the prM and in the PL in the PO. Activation maps between CS+ un. trials and aversive learning showed some similarities. Cluster details can be seen in Tab. 14.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	9982	7.66	-0.34	-78.4	-0.62	L	74% Lingual Gyrus
	1575	4.56	52.6	-42.8	37.5	R	58% Supramarginal Gyrus posterior division 71% Inferior parietal lobule
	1040	5.18	2.8	18.1	48.6	R	82% Cingulate Gyrus anterior division 60% Paracingulate Gyrus 40% Premotor Cortex
	1004	4.18	44	8.87	41.7	R	55% Precentral Gyrus
	577	3.83	0.947	-76.8	48.2	R	45% Precuneous Cortex
	576	5.39	39.9	24.2	-1.14	R	49% Insular Cortex 45% Frontal Operculum Cortex 32% Frontal Orbital Cortex
	363	4.04	-37.6	21.7	0.809	L	52% Insular Cortex 47% Frontal Operculum Cortex
CS-	21722	7.67	-0.31	-74.6	5.79	L	61% Thalamus 35% Parahippocampal Gyrus posterior division 51% Lingual Gyrus 45% Hippocampus subiculum
	341	3.42	31.5	53.2	20.6	R	75% Frontal Pole
	327	3.32	-54.5	-52.5	25.1	L	46% Angular Gyrus 36% Inferior parietal lobule
US	1670	4.49	-54.3	-14.6	18.9	L	39% Parietal Operculum Cortex 77% Secondary somatosensory cortex / Parietal operculum
	1618	4.62	30	-17.2	66.8	R	51% Precentral Gyrus 91% Premotor Cortex
	1548	4.2	-37.6	-86.2	9.48	L	49% Occipital Pole
	1518	3.92	50.4	-4.17	11.4	R	36% Central Opercular Cortex
	538	3.82	26.5	-92.6	1.23	R	43% Occipital Pole
	348	3.97	7.95	-20.3	28.4	R	43% Cingulate Gyrus
	347	3.98	1.49	-38.5	7.41	R	33% Cingulate Gyrus
	335	3.72	-6.98	-39.1	43.8	L	32% Precuneous Cortex, 32% Cingulate Gyrus posterior division
	309	3.44	45.9	13.4	28.3	R	33% Middle Frontal Gyrus

	835	4.37	3.3	11.9	49.2	R	76% Cingulate Gyrus anterior division 43% Paracingulate Gyrus
CS+ un. > CS-	545	3.81	59.1	-35.5	33.6	R	48% Parietal Operculum 30% Supramarginal Gyrus anterior division 60% Premotor Cortex
	308	5.24	-1.94	-85.4	-4.95	L	42% Lingual Gyrus

Table 14: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in SABP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the biggest cluster in the LG and a number of clusters covering bilateral IC, the supramariginal gyrus, ACC and prG. Processing of the CS- activated 3 clusters in frontal, parietal and occipital brain regions including the parahippocampal gyrus. US activation involved the highest numbers of significant clusters, activating mainly sensory brain areas, such as the S2, PO and prG. The aversive learning contrast between CS+ un. > CS- showed a significant increase in BOLD response in 3 clusters activating frontal and posterior parts of the brain. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

CBP patients showed a significant BOLD increase for the processing of the CS+ un., CS-, aversive US and for the aversive learning contrast during ACQ1 (Fig. 16 and Tab. 15). BOLD responses during CS+ un. processing and for the aversive learning contrast showed similar activation patterns in the Tha, middle frontal, precentral and in the anterior cingulate gyri, with CS+ un. trials showing significant activation in five clusters and aversive learning in four significant clusters (Tab. 15). In addition, CS+ un. trials also activated the IC bilateral (Fig. 16A). CS- trials only activated one significant cluster in the OL (Fig. 16B). Aversive US processing showed a significant increase in BOLD responses in five clusters within the brain, covering both hemispheres in the poG, prG, PO, the IC, S2 besides the prM and the OL (Fig. 16C and Tab. 15).

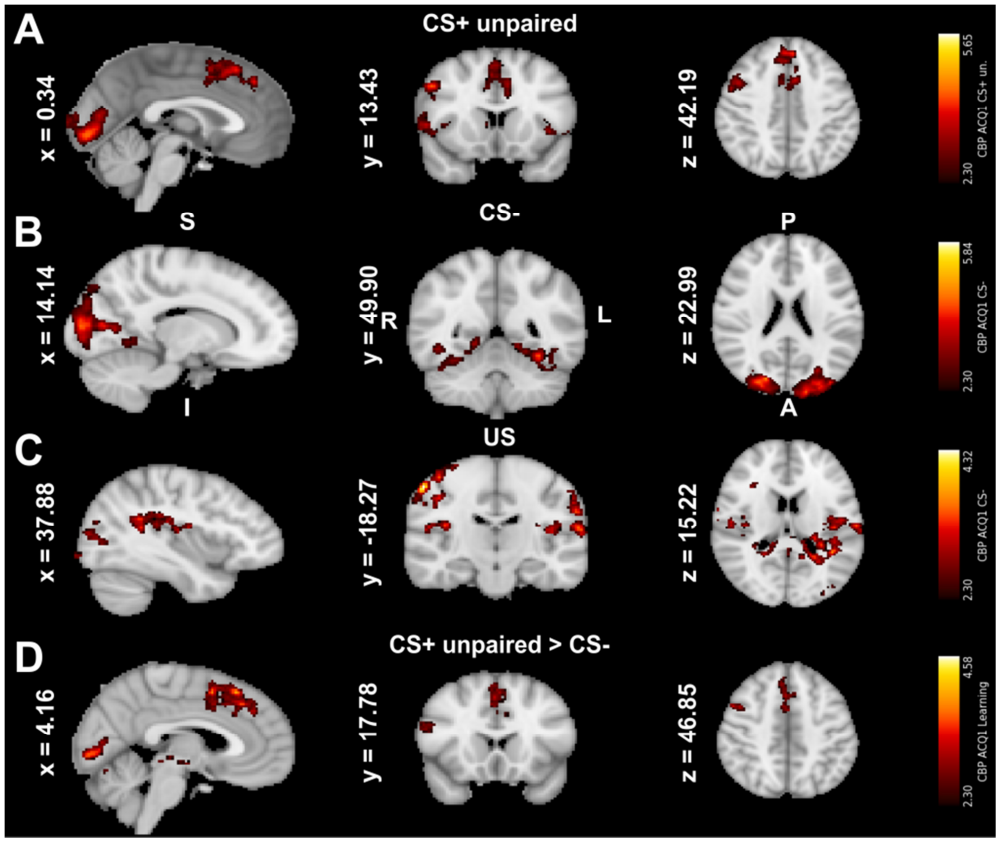

Figure 16: Trial-averaged BOLD response during aversive conditioning in CBP patients in ACQ1 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of CBP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 5 clusters involving the bilateral IC, the VC and the middle frontal, precentral and anterior cingulate gyri. These significant activation patterns were comparable to the activity maps during aversive learning (D), except for the bilateral IC activation which was only found in CS+ un. trials. (A). **B:** CS- trials activated 1 significant cluster in the occipital pole. **C:** US stimulation showed significant BOLD activation which was covering both hemispheres in the IC, S2, PO, prG, prM and poG. Cluster details can be seen in Tab. 15.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	4515	5.65	-4.58	-82.1	2.01	L	51% Intracalcarine Cortex 46% Lingual Gyrus 46% Visual cortex V1 BA17
	1553	4.32	46.6	16	13	R	66% Insular Cortex 50% Middle Frontal Gyrus 47% Precentral Gyrus
	1143	4.24	1.32	20.1	47.5	R	88% Cingulate Gyrus anterior 39% Paracingulate Gyrus 40% Premotor Cortex
	357	4.45	-38.6	18.5	0.11	L	48% Insular Cortex
	321	3.84	13.1	-5.77	6.13	R	100% Thalamus
CS-	8660	5.84	-0.011	-79.6	8.68	L	49% Occipital Pole
US	3408	3.94	-23.1	-37.6	19	L	69% Left Cerebral Cortex 66% Insular Cortex 58% Postcentral Gyrus 46% Insular Ig2 69% Secondary somatosensory cortex / Parietal operculum
	1878	4.32	46.4	-5.48	44.1	R	64% Postcentral Gyrus 53% Precentral Gyrus 80% Premotor Cortex
	624	3.77	-30.8	-89.8	1.42	L	61% Occipital Pole
	487	3.71	32.8	-90.4	-4.19	R	53% Occipital Pole
	430	3.43	53	-11.9	14.3	R	75% Insular Cortex 46% Central Opercular Cortex 60% Secondary somatosensory cortex / Parietal operculum
CS+un. > CS-	875	4.58	1.53	-81.8	-3.87	R	49% Lingual Gyrus 54% Visual cortex V2 BA18
	765	4.23	1.84	21	45.6	R	88% Cingulate Gyrus anterior 62% Paracingulate Gyrus division
	368	3.39	47.1	9.69	38.2	R	42% Middle Frontal Gyrus 40% Precentral Gyrus 30% Premotor cortex
	323	3.43	5.52	-10.7	-2.51	R	90% Thalamus

Table 15: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in CBP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the 7 clusters sharing similar brain responses as in the learning contrast, showing both significant activation in the IC, the middle frontal and the paracingulate gyri. In addition, the CS+ un. showed significant clusters in the VC and in the Tha. Aversive US processing involved the highest number of significant clusters activating both hemispheres in the poG, prG, the IC, S2 and the occipital pole. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

3.2.2 Whole-brain analyses of aversive learning-related brain activation within subject groups during ACQ2

HC showed significant BOLD responses for all three stimuli presented during ACQ2 in the aversive conditioning paradigm (Fig. 17A-C). Furthermore, the aversive learning contrast, between CS+ un. > CS-, elicited significant brain responses (Fig. 17D and Tab. 16). BOLD response activation patterns during CS+ un. processing activated eight significant clusters, covering both hemispheres, including the IC and the posterior supramarginal gyrus, PO, Tha, PL (Fig. 17A). Activated clusters in the right hemisphere were always bigger than in the left hemisphere (Tab. 16). In contrast to that, CS- processing activated three significant clusters only in the right hemisphere including the IC, the prG and parts of OL (Fig. 17B). Processing of the aversive US activated mostly brain areas in the right hemisphere, such as the S1, PO prM, primary motor cortex (M1) and the poG (Fig. 17C). Aversive learning involved the right Tha, the PL and the OL (Fig. 17D and Tab. 16).

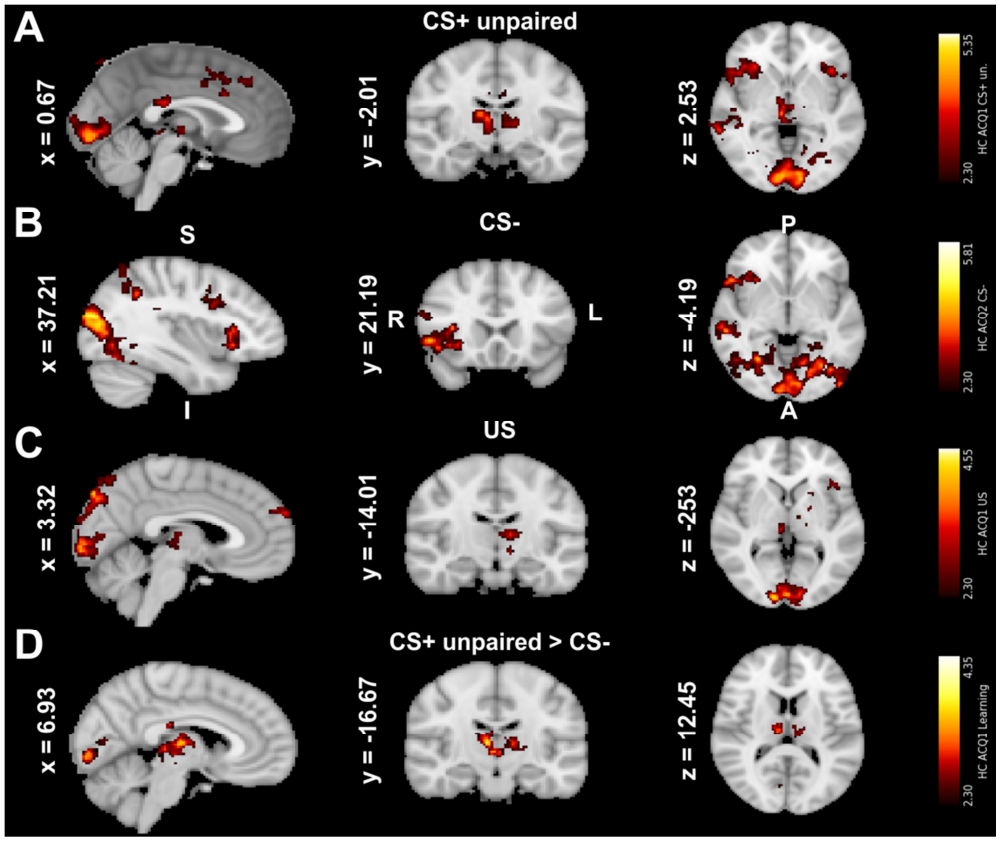

Figure 17: Trial-averaged BOLD response during aversive conditioning in HC in ACQ2 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of HC, , cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars depict Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 8 clusters involving

the IC bilaterally, the supramarginal gyrus, the pCC and the Tha. **B:** CS- trials activated similar brain regions as during CS+ un. processing, activating 3 significant clusters only in the right hemisphere of the brain, except for the OL, the IC and the prG. **C:** US stimuli activated only significant clusters in the right hemisphere, including the prM, primary motor cortex (M1), poG, PO and the ACC. **D:** The aversive learning contrast mainly activated the right Tha and the posterior part of the supramarginal gyrus. Cluster details can be seen in Tab. 16.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	7371	5.02	-2.1	-79.7	2.86	L	44% Occipital Pole
	1990	5.35	44	17.3	16.7	R	56% Middle Frontal Gyrus 55% Insular Cortex
	1816	4.3	51.9	-44.8	36.0	R	56% Supramarginal Gyrus posterior division 31% Parietal Operculum
	1454	4.86	2.75	-18.7	11.9	R	100% Thalamus
	462	3.98	3.18	17.6	46.4	R	90% Cingulate Gyrus posterior division
	448	4.22	57.4	-32.9	-4.67	R	56% Middle Temporal Gyrus posterior division
	435	3.9	-36.5	21.4	-0.22	L	57% Insular Cortex 35% Frontal Orbital Cortex
	339	3.4	-56	-48.8	25.8	L	49% Supramarginal Gyrus posterior division 44% Inferior parietal lobule PFm
CS-	14264	5.81	5.5	-75.6	13.4	R	60% Lateral Occipital Cortex superior division
	698	4.37	42	22	-0.78	R	49% Insular Cortex
	553	4.72	45.9	8.81	34.3	R	45% Precentral Gyrus
US	1610	4	51.2	-16.4	14.4	R	41% Parietal Operculum
	1407	5.22	43.5	-19.1	57.2	R	67% Postcentral Gyrus 74% Primary somatosensory cortex 68% Premotor Cortex 53% Primary motor cortex
	341	3.86	2.69	0.475	41.9	R	79% Cingulate Gyrus anterior division 50% Premotor Cortex
	248	3.56	-42.1	-74.6	8.57	L	67% Lateral Occipital Cortex
CS+un. > CS-	1135	4.35	-3.94	-81.5	-8.93	L	77% Lingual Gyrus
	544	3.73	2.31	-18.6	8.31	R	100% Thalamus
	335	3.32	53.1	-45	39.3	R	46% Supramarginal Gyrus posterior division 50% Inferior parietal lobule PFm

Table 16: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in HC. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the highest number of clusters, whereas the CS- processing activated the largest cluster. Both, activating the IC in CS+ un. trials bilaterally and in the right hemisphere in CS- trials. US activation could be found in only in the right hemisphere with

significant BOLD responses in S1, M1, PO, prM, poG and the OL. Aversive learning showed a significant increase in BOLD responses in the right Tha and the posterior part of the supramarginal gyrus. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

SABP patients showed significant brain activation patterns for the CS+ un., the CS- and the US processing (Fig. 18A-C) during ACQ1 and for the aversive learning contrast (Fig. 18D and Tab. 17). CS+ un. showed significant BOLD responses in five clusters including the IC bilaterally, frontal brain areas, as well as occipital parts of the brain (Fig. 18A). In contrast to that, the CS- elicited significant activation patterns only in one cluster in the OL (Fig. 18B). US BOLD responses activated four clusters, with several clusters covering both hemispheres including the S1, S2, prM, poG, prG with stronger activation in the right hemisphere, contralateral to the stimulation site (Fig. 18C). The aversive learning contrast activated four clusters covering frontal, parietal and occipital brain areas (Fig. 18D and Tab. 17).

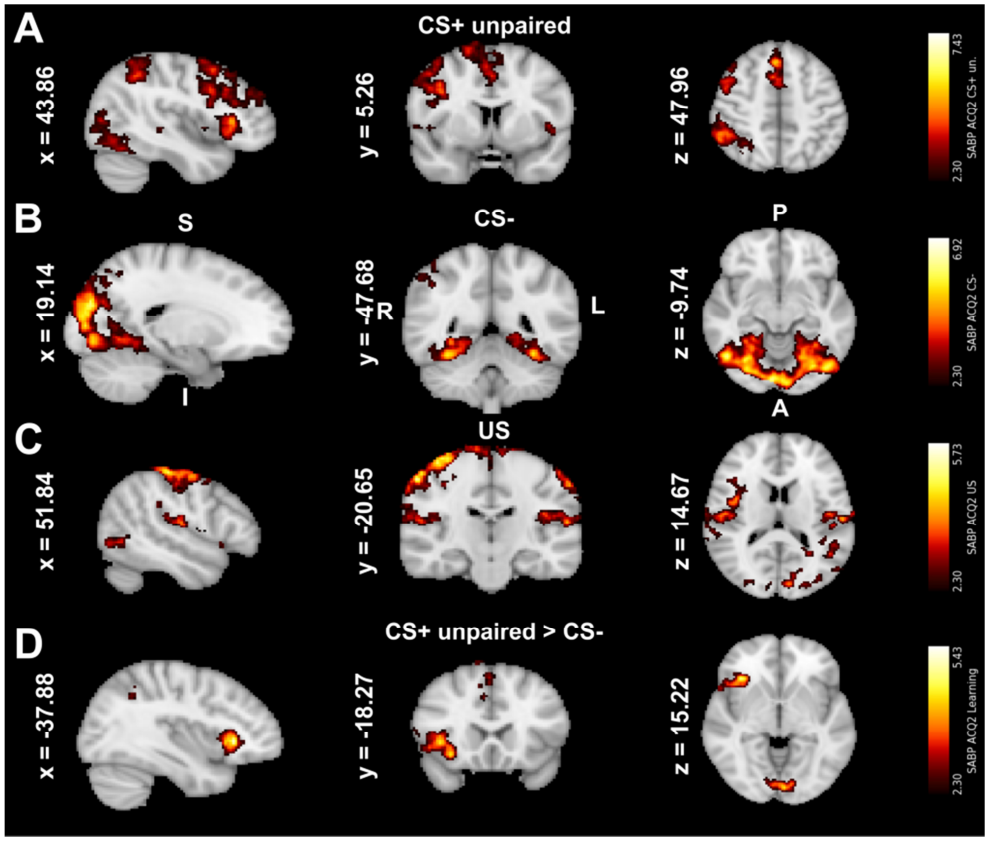

Figure 18: Trial-averaged BOLD response during aversive conditioning in SABP patients in ACQ2 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of SABP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with

red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** CS+ un. trials activated 5 clusters involving the IC, prG, the PL and the occipital pole. **B:** CS- trials activated 1 significant cluster in the OL. **C:** US stimulation showed significant BOLD activation in 5 clusters covering both hemispheres, with stronger activation in the right hemisphere, including the S1, S2, M1, PO, prG, poG and the OL. **D:** Aversive learning-related brain activation could be found in frontal, parietal and occipital parts of the brain. Cluster details can be seen in Tab. 17.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	11148	7.43	0.49	-77.4	-0.10	R	64% Occipital Pole
	3830	5.77	44.1	20.8	22.2	R	70% Insular Cortex 30% Precentral Gyrus
	2365	4.95	49.7	-43.8	37	R	36% Angular Gyrus 86% Inferior parietal lobule
	2169	5.41	4.31	14.8	54.9	R	67% Superior Frontal Gyrus
	853	4.72	-38.6	20.5	-0.15	L	48% Insular Cortex 31% Frontal Operculum Cortex
CS-	9201	6.92	3.55	-75.2	7.45	R	50% Occipital Pole
US	4553	5.73	41.1	-15.1	47.0	R	70% Postcentral Gyrus 51% Precentral Gyrus 55% Premotor Cortex 54% Primary somatosensory cortex 40% Primary motor cortex 48% Secondary somatosensory cortex / Parietal operculum
	4142	4.37	-31.4	-63.7	6.03	L	44% Occipital Fusiform Gyrus
	2063	4.46	31.5	-76.8	-3.04	R	48% Occipital Fusiform Gyrus
	832	4.27	-51	-24.9	55.4	L	66% Postcentral Gyrus 70% Secondary somatosensory cortex / Parietal operculum 90% Primary somatosensory cortex
CS+un. > CS-	829	5.43	40.2	23	-0.99	R	59% Frontal Operculum Cortex 57% Insular Cortex
	602	4.39	-2.24	-83.9	-1.81	L	52% Lingual Gyrus
	597	4.55	3.42	23.3	50.7	R	64% Superior Frontal Gyrus
	427	3.51	49.4	-48.3	48.8	R	51% Angular Gyrus 80% Inferior parietal lobule

Table 17: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in SABP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the biggest cluster in the IC bilaterally and the right PL. Processing of the CS- activated only 1 cluster in the occipital pole. Aversive US processing showed significant BOLD responses bilaterally in the S1, S2, M1, PO, prG and poG with stronger activation of the right hemisphere contra lateral to the stimulation site. The aversive learning showed a

significant increase in BOLD responses in 4 clusters in the IC and OFC bilaterally and the right PL. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

CBP patients showed a significant BOLD increase for the processing of the CS+ un., CS-, aversive US (Fig. 19A-C) and for the aversive learning contrast during ACQ2 (Fig. 19D and Tab. 18). BOLD responses during CS+ un. processing and for the aversive learning contrast showed similar activation patterns, in the middle frontal and in the paracingulate gyri, the ACC, the IC and the putamen (Put). CS+ un. trials activated six clusters in both hemispheres (Fig. 19A), whereas aversive learning showed four significant clusters in the right hemisphere (Fig. 19D). CS- trials activated only one significant cluster in the OL (Fig. 19B). Aversive US processing showed a significant increase in BOLD responses in two significant clusters covering poG, prG, M1 and S1 (Fig. 19C and Tab. 18).

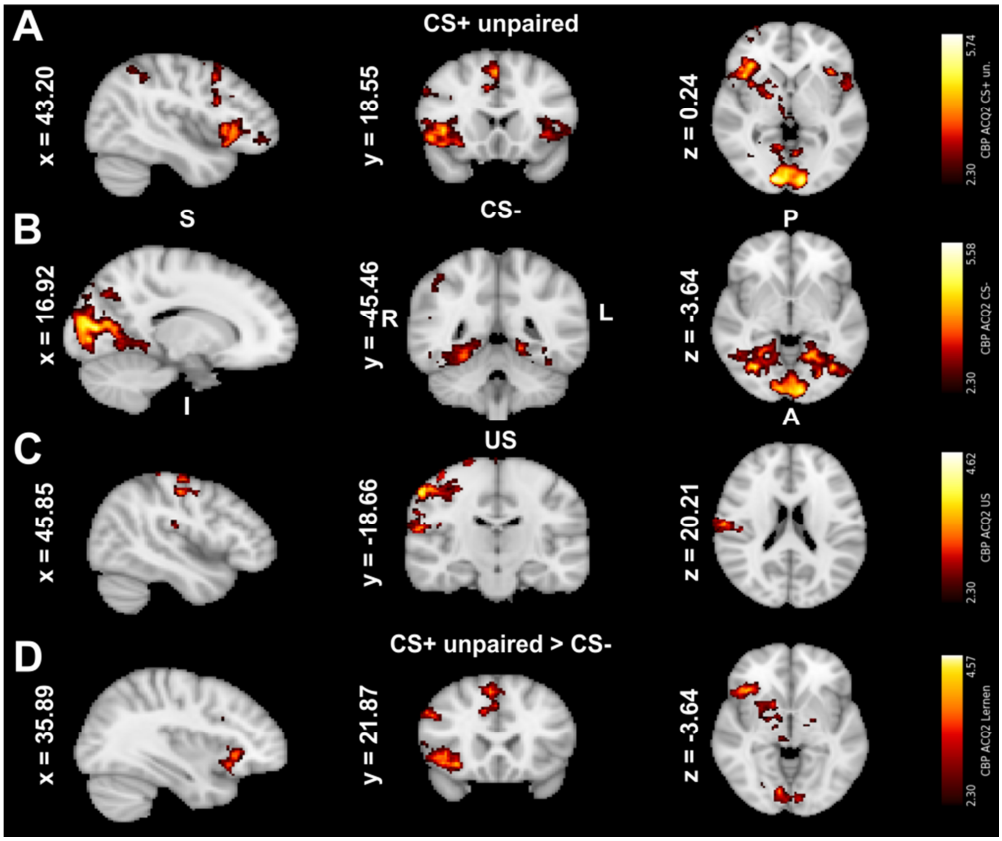

Figure 19: Trial-averaged BOLD response during aversive conditioning in CBP patients in ACQ2 (A) during CS+ un., (B) CS-, (C) US processing and (D) learning-related brain activation, respectively. fMRI whole-brain data of CBP patients, cluster corrected ($p < .05$), threshold at $Z > 2.3$ (Flame 2). Brain Structures were identified using the Harvard-Oxford Cortical Structural Atlas. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. **A:** The CS+ un. involved significant

BOLD increases in 6 clusters which were comparable to brain responses in the aversive learning contrast (D), showing both significant activation in the putamen (Put), the IC and the ACC, as well as in frontal and parietal parts of the brain. **B:** The CS- trials showed significant BOLD responses in 1 cluster in the occipital pole. **C:** Aversive US processing involved 2 significant clusters, activating both hemispheres in the poG, prG, S1, M1. Cluster details can be seen in Tab. 18.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
CS+ un.	5465	5.74	0.674	-78.6	1.34	R	53% Lingual Gyrus
	2088	5.02	34.7	20.3	-1.3	R	79% Insular Cortex 68% Frontal Orbital Cortex 99% Putamen
	900	5	3.49	23.3	44.5	R	70% Paracingulate Gyrus 43% Cingulate Gyrus anterior division
	638	3.6	45.5	11.7	43.1	R	52% Middle Frontal Gyrus 32% Premotor cortex
	518	4.2	-42.7	16.1	0.66	L	63% Insular Cortex 56% Frontal Orbital Cortex
	510	3.35	50.6	-44.9	40.6	R	45% Angular Gyrus 57% Inferior parietal lobule
CS-	13545	5.58	2.31	-73.9	8.6	R	54% Occipital Pole
US	1273	4.62	48.5	-18.8	47.0	R	67% Postcentral Gyrus 56% Primary somatosensory cortex
	337	3.32	-2.18	-22.6	76.1	L	52% Precentral Gyrus 47% Premotor 30% Primary motor cortex
CS+un. > CS-	1720	4.37	23.5	5.83	-2.78	R	96% Putamen 70% Insular Cortex
	809	4.57	3.81	24.2	45.2	R	71% Paracingulate Gyrus 34% Cingulate gyrus anterior division
	406	3.69	47.8	14.8	37.8	R	37% Precentral Gyrus 44% Inferior parietal lobule 40% Primary somatosensory cortex
	374	3.92	6.10	-83.7	-3.90	R	60% Lingual Gyrus

Table 18: Cluster list of trial averaged whole-brain BOLD responses during CS+ un., CS-, US processing and in the learning contrast in CBP patients. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. The CS+ un. involved the 6 clusters sharing similar brain responses as in the learning contrast, showing both significant activation in the IC, Put, the ACC and in frontal and parietal parts of the brain. The CS- showed only a significant increase in BOLD responses in the OL. Aversive US processing involved both hemispheres including poG, prG, S1, M1 and the prM. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

Hypothesis 2: Heightened aversive learning responses in pain patients

3.2.3 Group comparison of aversive learning-related brain activation in ACQ1

In sections 3.2.1 and 3.2.2 the underlying brain processes during aversive conditioning and the used stimuli were discussed, within each group, respectively, to provide a basis for the group comparisons. In this section focus will be laid on learning-related BOLD responses between both pain patient groups and both patient groups in comparison to HC. In contrast to the appetitive conditioning data (section 3.1), the statistical threshold of the cluster correction had to be adjusted, since data did not always reach the significance threshold ($Z = 2.3$, $p < 0.05$). Only the group comparison between SABP and CBP patients resulted in a significant group contrast (whole-brain data) in which the cluster threshold ($Z = 2.3$, $p < 0.05$) was not adjusted. SABP patients showed lower BOLD responses in the OL when compared to patients with CBP during aversive learning (Fig. 20B and Tab. 19). Since no more significant clusters could be found in neither whole-brain data nor in the ROI analyses in both acquisitions. The cluster threshold was lowered in the analyses to $Z = 2.0$ with a significance threshold $p = 0.05$. Clusters which reached Z threshold = 2.0 were considered as significant, if this threshold was not reached, data were not considered. After cluster threshold adjustments significant group differences were seen between HC and patients with SABP (whole-brain data). HC showed a stronger increase of BOLD responses in the OL during acquisition of the aversive CS+ when compared to patients with SABP (Fig. 20A and Tab. 19).

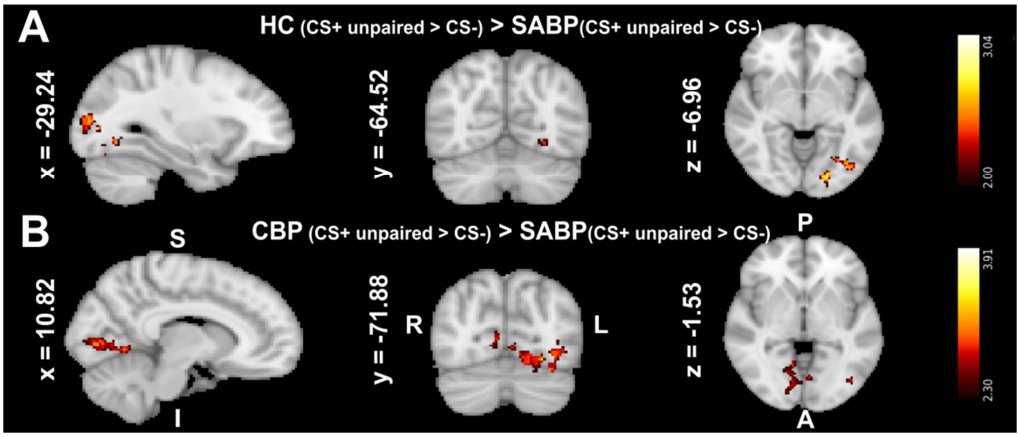

Figure 20: Brain activity maps for aversive learning (contrast CS+ un. > CS-) during ACQ1. BOLD responses (whole-brain) during aversive learning in the group contrast (A) HC > SABP and (B) CBP > SABP. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars depict Z-values with strongest activations in yellow and lower activations in red. **A:** Cluster threshold for the group contrast between HC and SABP patients was adjusted to Z = 2.0 at a significance level p = .05. HC showed stronger activation of the left occipital fusiform gyrus when compared to

patients with SABP. **B:** CBP patients showed significant learning-related brain activation in the LG for the acquisition of the aversive CS+ un. > CS- in contrast to SABP patients (cluster threshold Z = 2.3).

ROI analyses showed only significant learning-related group differences in the data with the lowered cluster threshold (Z = 2.0). The IC mask resulted in most prominent findings, showing divergent activation patterns within the IC between subject samples (Fig. 21 and Tab. 19). HC activated a small cluster in the left site of the posterior IC in contrast to patients with CBP (Fig. 21A). CBP patients activated slightly bigger clusters in both anterior and posterior parts of the IC, during aversive learning. In addition, they showed two significant clusters in the left portion of the IC with one cluster shifted more to the posterior and the other one to the anterior part of the IC when compared to HC (Fig. 21B). CBP patients elicited stronger activation in the anterior parts of the left IC during aversive learning when contrasted to SABP patients (Fig. 21C). Stronger involvement of the posterior part of the left IC was seen in SABP patients in contrast to CBP patients (Fig. 20D). Only the group contrast between HC and SABP patients did not yield any significant differences in the IC during aversive learning. Group comparison between the HC sample and patients with SABP resulted in significant differences in the ACC cortex, showing stronger BOLD responses in SABP patients than in HC (Fig. 21E).

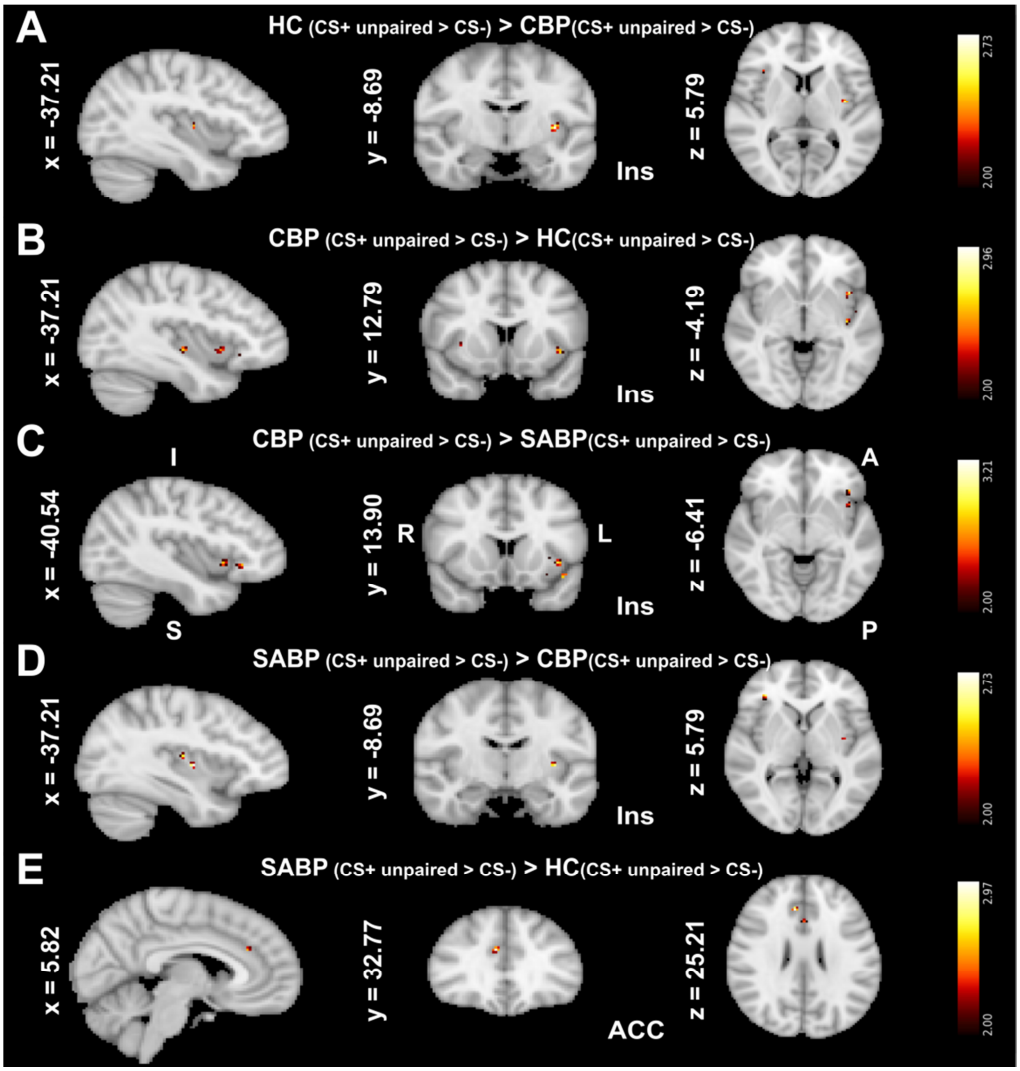

Figure 21: Brain activity maps for aversive learning (contrast CS+ un. > CS-) during ACQ1 in ROI. Group contrast between (A) HC > CBP, (B) CBP > HC, (C) SABP > CBP (D) and CBP > SABP in the IC. (E) Group contrast between SABP > HC in the ACC. Brain masks used in the ROI analyses were selected from the Harvard-Oxford Cortical Structural Atlas. Used masks were covering both sites of the respective brain area. X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. The IC mask revealed most prominent results in almost all group comparisons in the ROI analyses, except for the contrast between HC and patients with SABP, in the cluster corrected data (Z = 2.0). All clusters with a threshold lower than Z = 2.0 were excluded. **A:** HC showed significant learning-related brain activation in the left IC in contrast to patients with CBP. **B:** CBP patients revealed slightly bigger clusters in the IC, 2 in the left IC and 1 cluster in the right IC when compared with HC. **C:** CBP patients showed learning-related BOLD responses in the anterior part of the IC when contrasted to SABP patients. **D:** SABP patients showed stronger activity in the right posterior IC in contrast to CBP patients. **E:** Aversive learning contrast between SABP patients and HC resulted in stronger activation of the ACC in SABP patients when contrasted to HC. Cluster details can be seen in Tab. 19.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
HC > SABP (whole- brain)	524	3.04	-29.6	-80.1	0.12	L	38% Occipital Fusiform Gyrus
CPB > SABP (whole-brain)	670	3.91	-11.8	-73	-5.4	L	45% Lingual Gyrus 53% Visual cortex
SABP > HC (ACC)	10	2.97	7.62	32.6	26.4	R	45% Cingulate Gyrus anterior division 43% Paracingulate Gyrus
HC > CBP (IC)	13	2.73	-35.7	-7.4	4.76	L	100% Insular Cortex
	4	2.26	36.5	23.5	5.01	R	50% Frontal Operculum Cortex
CBP > HC (IC)	16	2.77	-39.3	12.2	-4.38	L	73% Insular Cortex
	10	2.62	-38.8	-15.4	-4.18	L	52% Insular Cortex
	9	2.96	34.2	9.95	1.09	R	39% Insular Cortex
SABP > CBP (IC)	15	2.6	39.6	-4.3	-2.31	R	70% Insular Cortex
	12	2.63	34.8	34.6	0.96	R	52% Frontal Operculum Cortex
	11	2.73	-34.9	-8.57	4.33	L	36% Insular Cortex
CPB > SABP (IC)	21	3.21	-43.7	11.2	-13.2	L	93% Insular Cortex 31% Temporal Pole
	13	2.89	-38.9	14.0	-4.30	L	75% Insular Cortex 32% Insular Id1
	11	2.86	36.5	6.91	-9.33	R	58% Insular Cortex

Table 19: Cluster list of increased BOLD responses during aversive learning. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. Whole-brain data depicted in the upper panel. ROI analyses data can be seen in the lower panel of the table. Whole-brain data resulted in significant learning responses in the OL in HC when contrasted to SABP patients (Z = 2.0). A significant cluster in SABP patients was activated in the VC in contrast to patients with CBP (Z = 2.3) in the whole-brain data. ROI analyses showed in almost all group contrasts significant BOLD responses in the IC during aversive learning, eliciting divergent activation patterns between the different subject samples. Only the comparison between HC and SABP patients did not show any significant difference in IC activation. SABP patients activated the ACC more strongly than HC during aversive leaning. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

3.2.4 Group comparison of aversive learning-related brain activation in ACQ2

There was only a significant group difference between HC and patients with CBP, during ACQ2 in the whole-brain data. HC showed stronger activation in the pCC, the IC, parts of the poG, S1 and in the temporal gyrus, during the acquisition of the aversive CS+ when compared to CBP patients (Fig. 22 and Tab. 20). In contrast to ACQ1 also the lowered cluster threshold failed to produce significant results in ROI.

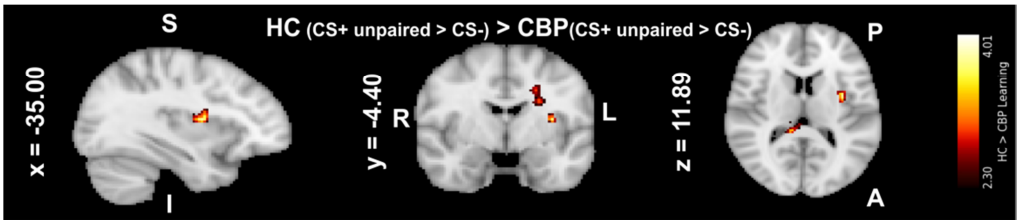

Figure 22: Brain activity maps for aversive learning (contrast CS+ un. > CS-) during ACQ2. Group contrast between HC > CBP (whole-brain). X, y and z coordinates are in MNI space, corresponding to anterior-posterior (A, P), inferior-superior (I, S) and left-right axes (L, R). Activation maps are plotted onto a MNI152 standard-space T1-weighted average structural template image. Voxels depicted with red and yellow colour represent Z-values of clusters with a significantly increased BOLD response. Colour bars represent Z-values with strongest activations in yellow and lower activations in red. HC showed stronger activation in the IC, pCC, poG and the temporal gyrus than CBP patients during aversive learning. Cluster details can be seen in Tab. 20.

Stimulus	*Number of Voxels*	*Peak Z*	*X*	*Y*	*Z*	Hemi.	*Brain Structure*
HC > CBP (whole-brain)	329	4.01	7.65	-33.1	24.3	R	40% Cingulate Gyrus posterior division
	316	3.78	-26.7	-7.85	26.2	L	45% Insular Cortex 34% Postcentral Gyrus 65% Primary somatosensory cortex
	308	3.37	56.6	-39.5	-14.2	R	51% Inferior Temporal Gyrus 30% Middle Temporal Gyrus

Table 20: Cluster list of increased BOLD responses during aversive learning in the group comparison HC > CBP. All depicted values were estimated for the location of the peak voxel within a given cluster. Harvard-Oxford Cortical Structural Atlas and the Juelich Histological Atlas were used for the assignment of the peak to a certain brain area and its probability. Whole-brain data resulted in significant learning responses in the left IC, poG, pCC, S1 and in the temporal gyrus in HC when contrasted to CBP patients. There were no other significant group comparisons in ACQ2. Stimulus: mean stimulus, Number of Voxels = number of voxels activated in the given cluster, Peak Z = maximum threshold of activated voxels, X, Y, Z = MNI coordinates of the peak voxel, Hemi. = Hemisphere (R, L).

Hypothesis 3: Impaired aversive sensory processing in back pain patients

3.2.5 Interaction between fMRI data and behavioural responses

The previous sections (section 3.2.3-3.2.4) indicated that learning mechanisms were less distinct between subject samples, during aversive conditioning than during appetitive learning. The IC showed most prominently divergent activation patterns during aversive learning between subject samples. The group contrast between HC and patients with SABP did not show divergent learning-related brain activation patterns in the IC, but in the ACC. SABP patients showed a stronger involvement of the ACC during aversive learning than HC. To test whether the aversive US evaluation, on a behavioural level, as well as the physiological responses elicited by the painful sensory stimulation, had an impact on those divergent BOLD responses during learning, the US responses during the HAB were considered in the analyses (for more details see sections 3.1.5-3.1.6).

A significant correlation between US valence ratings and learning-related responses in different ROIs could only be found in the HC sample. Which showed a negative, but significant correlation between the aversive US valence ratings during HAB and aversive learning responses in the ACC ($r(37) = -.438$, $p = .009$), dlPFC ($r(37) = -.450$, $p = .006$) and the S2 ($r(37) = -.402$, $p = .015$), during ACQ1. Furthermore, in ACQ2 a negative significant correlation could be seen again in the ACC ($r(37) = -.468$, $p = .004$) and the S2 ($r(37) = -.345$, $p = .039$), but also in the Amy ($r(37) = -.399$, $p = .014$), the Hipp ($r(37) = -.434$, $p = .007$), the NAC ($r(37) = -.350$, $p = .034$) and the OFC ($r(37) = -.377$, $p = .022$) (Fig. 23 and Tab. 21).

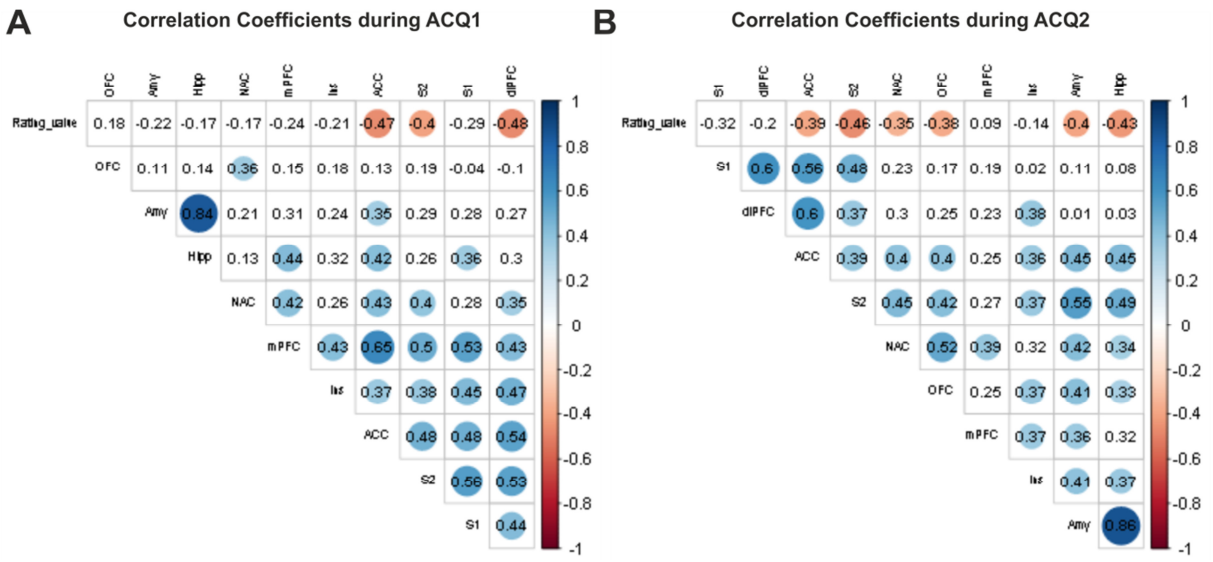

Figure 23: Correlation matrix: US valence ratings and learning-related BOLD responses in HC. Spearman correlation (two-tailed) was calculated between BSC during appetitive learning (CS+ un. > CS-) during (A) ACQ1 and (B) ACQ2 in different ROIs and US valence ratings in the HAB. Spearman correlation was chosen to account

for the non-normally distributed data of the US valence rating data in the HAB. Correlation coefficients (Spearman's rho) are depicted in the first row of the correlation matrix (Rating_value). Significant correlations are highlighted with coloured circles, in blue for a positive correlation and in red for a negative correlation. Circle size represents significance thresholds with $p < .05$ (small circle), $p < .01$ (medium circle) and $p < .001$ (large circle) with corresponding significance levels depicted on the colour bar. **A-B:** HC showed a negative correlation between US valence ratings and aversive learning responses in both acquisitions in the ACC and S2. Additionally, there were significant negative correlations in the dlPFC (ACQ1), the Amy, Hipp, NAC and the S2 (ACQ2). Numbers = Spearman's rho, circles = significance level, Amy = amygdala, Hipp = hippocampus, NAC = nucleus accumbens, IC = insular cortex, mPFC = medial prefrontal cortex, S2 = secondary somatosensory cortex, ACC = anterior cingulate cortex, dlPFC = dorsolateral prefrontal cortex, = orbitofrontal cortex, S1 = primary somatosensory cortex. Correlation coefficients are depicted also in Tab. 21.

Correlation Coefficients

group	*Phase*	*ROI*	*statistics*		*df*
HC	ACQ1	ACC	r	-0.468 **	37
			p	0.004	
		dlPFC	r	-0.450 **	
			p	0.006	
		S2	r	-0.402 *	
			p	0.015	
HC	ACQ2	ACC	r	-0.389 *	37
			p	0.017	
		Amy	r	-0.399 *	
			p	0.014	
		Hipp	r	-0.434 **	
			p	0.007	
		NAC	r	-0.350 *	
			p	0.034	
		OFC	r	-0.377 *	
			p	0.022	
		S2	r	-0.345 *	
			p	0.039	

Table 21: Correlation coefficients: US valence ratings and learning-related BOLD responses during aversive learning. Spearman correlation coefficients were calculated between learning-related brain activation in different ROI during aversive conditioning (ACQ1 and ACQ2) and US valence ratings in the HAB, within each group, respectively. Spearman correlation was chosen to account for the non-normally distributed data of the US valence rating data in the HAB. HC showed a significant but negative correlation between US valence ratings and learning responses in both acquisitions. The ACC and the S2 showed in both acquisitions a significant negative correlation with valence ratings of the painful stimulation during HAB and learning-related BOLD responses. In addition, a negative correlation could be seen also in dlPFC during ACQ1 and in the Amy, Hipp, NAC and OFC during ACQ2. r = Spearman's rho, p = significance level, df = degrees of freedom, Asterisks depict significant correlations (*$p < .05$, **$p < .01$). ACC = anterior cingulate cortex, Amy = amygdala, dlPFC = dorsolateral prefrontal cortex, Hipp = hippocampus, NAC = nucleus accumbens, OFC = orbitofrontal cortex, S2 = secondary somatosensory cortex.

3.2.6 Correlation between fMRI data during painful stimulation and aversive learning responses

In a second step, a linear regression model was used to calculate the association between the BOLD responses during painful electrical stimulation in the HAB and learning-related responses in the ROI data. The regression model was used to test whether the physiological response during an aversive sensory stimulation could predict the associated learning-related responses in all subject groups. The regression analyses revealed a significant relationship between brain activation during painful stimulation and learning-related brain activation in all subject samples. A significant association between ROI activation during pain stimulation and learning-related brain activation was found in HC and CBP patients in ACQ1 and ACQ2. HC showed a significant negative relationship in the mPFC ($F(1,37) = 8.436$, $p = .006$) and in the S2 ($F(1,37) = 7.407$, $p = .010$) during ACQ1 and a positive relationship in the IC ($F(1,37) = 4.857$, $p = .034$) and in the NAC ($F(1,37) = 4.992$, $p = .003$) during ACQ2. Learning-related BOLD signal changes showed a significant dependency on brain responses during US processing in the S1 ($F(2,34) = 10.850$, $p = .002$) during ACQ1 in CBP patients. During ACQ2 15 % of the shared variance during aversive learning were explained by the initial US response in the ACC ($F(2,34) = 5.686$, $p = .023$) and the NAC ($F(2,34) = 5.784$, $p = .022$). HC and patients with CBP, both, showed a significant association between US brain responses and aversive learning during ACQ2. Activation in the OFC ($F(1,47) = 8.261$, $p = .006$) explained 15 % of the variance in the learning-related brain responses during ACQ2 in SABP patients (Tab. 22).

Linear Regression								
group	***Phase***	***ROI***	***Unstandardized Coefficients***		***t***	***R^2***	***Adj. R^2***	***Sig.***
			B	***SE***				
HC	ACQ1	mPFC	-0.632	0.052	-2.904	0.194	0.171	0.006
		S2	-0.990	0.363	-2.722	0.175	0.151	0.010
CBP	ACQ1	S1	0.815	0.248	3.293	0.259	0.235	0.002
HC	ACQ2	NAC	0.646	0.289	2.234	0.125	0.100	0.032
		IC	0.807	-0.366	2.204	0.122	0.097	0.034
SABP	ACQ2	OFC	-0.517	0.180	-2.874	0.152	0.134	0.006
CBP	ACQ2	ACC	0.789	0.331	2.385	0.151	0.124	0.023
		NAC	0.435	0.181	2.405	0.153	0.127	0.022

Table 22: Linear regression model: BSC during aversive US during HAB and BSC during aversive learning. Dependent variable: learning-related BOLD responses during ACQ1 and ACQ2 in different ROIs. Predictors: Brain responses during aversive US stimulation during HAB. HC showed a significant negative relationship between US BOLD responses and the learning-related brain activation during ACQ1 in the mPFC, S2 and a positive during ACQ2 in NAC and the IC. CBP patients showed a dependency between aversive US BSC and learning-related responses during ACQ1 in the S1 and during ACQ2 in the ACC and NAC. Patients with SABP revealed a significant and negative association only during ACQ2 in the OFC. ACC = anterior cingulate cortex, IC = insular cortex, NAC = nucleus accumbens, mPFC = medial prefrontal cortex, OFC = orbitofrontal cortex, S1 = primary somatosensory cortex and S2 = secondary somatosensory cortex.

4. Discussion

The present thesis focuses on appetitive and aversive learning mechanisms and its maladaptation in different back pain stages. The study is based on fMRI acquisitions during appetitive and aversive conditioning in a group of HC, SABP and CBP patients. My data show different brain response patterns in appetitive and aversive learning within and between the subject samples.

4.1 Impaired appetitive learning in CBP

I hypothesized that appetitive learning is impaired in CBP patients, with a shift away from reward-related brain areas. My data support this assumption, by showing a shift away from limbic and striatal brain areas towards brain areas known to be involved in pain processing and cognition in both pain samples. Appetitive learning in SABP and CBP patients elicited different brain responses in contrast to the HC sample. Group comparisons of appetitive learning revealed stronger activation of striatal (NAC) and limbic brain areas (Hipp) in HC when compared to CBP patients (Fig. 10). My findings are in line with and extend previous studies on reward processing and appetitive learning. A study comparing activation within the NAC during conditioning, using an appetitive and an aversive CS, revealed that NAC responses increased during the appetitive CS and decreased during the presentation of the aversive CS (Gottfried, O'Doherty, and Dolan 2002). Several neuroimaging studies reported the involvement of the NAC in appetitive conditioning. The same studies showed that NAC activation represented the used US, after learning was established it reflected the CS+ which predicted the rewarding outcome (O'Doherty et al. 2003; O'Doherty et al. 2004; O'Doherty et al. 2006). The reduced NAC activation seen in CBP patients (Fig. 10) in the present study could thus represent a negative processing of the appetitive stimulus.

Further, I also found changes at the subacute pain level. Group comparisons revealed stronger activation in the Amy and Hipp in HC in contrast to patients with SABP (Fig. 12). Weaker Hipp activation was seen in both patient samples (Fig. 10 and Fig. 12). The Hipp was shown to be important for learning and memory processes in several studies (e.g. Ito et al. 2005). Moreover, it is known to be relevant for the acquisition of contingency awareness (Cacciaglia et al. 2015). Also, the Amy is a brain region commonly activated during conditioning and relevant for the emotional processing of the CS and the strength of CS-US association (Cacciaglia et al. 2015; Chase et al. 2015; Martin-Soelch, Linthicum, and Ernst 2007). The decreased Hipp activation seen in both, SABP (Fig. 12) and CBP patients (Fig. 10) thus indicates that appetitive learning is altered in contrast to HC. This is further supported by the reduced activity in the Amy in SABP patients (Fig. 12), strengthening the conclusion that emotional processing is altered in pain patients compared to HC. Reduced activation in limbic and striatal regions, as seen here in both pain groups, was also reported in stressed subjects during appetitive conditioning which was associated with lowered perception of the used US (food US) (Born

et al. 2010; Kruse et al. 2018). Decreased activation in the Hipp, Amy and NAC could reflect that the rewarding nature of the PT stimulation was diminished in both pain groups. Being in pain is a form of constant stress on the body which could explain the stronger activation in the Hipp, Amy and NAC in HC during appetitive learning when compared to the pain samples. Appetitive learning mechanisms were altered in both pain groups compared to HC. Depending on the pain state, those changes were different, with SABP patients showing reduced activation in the Hipp and Amy, whereas CBP patients revealed decreases in NAC and Hipp activation. Moreover, both pain patient groups indicated a shift towards pain-related brain areas in contrast to HC during appetitive learning. This was reflected by a stronger activation in the PO in SABP patients (Fig. 11A) and the pCC in CBP patients (Fig. 11B). The PO was recently discussed to be specific for the processing of painful heat stimuli and not reflecting saliency of the used stimulus (Horing, Sprenger, and Büchel 2019). Another study comparing masochists and HC could show that the PO dampened the motivational-affective aspects of painful sensations in the masochist group (Kamping et al. 2016). Moreover, a case study on a young woman with severe episodic pain, found a tumour in the PO (Potagas et al. 1997). These findings support the idea that the PO is involved in pain processing and sensations, rather than in pleasant sensations. I assume that the stronger activation seen in the PO in SABP patients could therefore reflect a form of sensitization, shifting the processing of the appetitive CS more towards pain. This could lead to allodynia in those subjects, feeling pain during non-painful stimulation. An over-representation of an innocuous sensory stimulation could pave the path for the development of chronic pain. Behavioural analyses did not show lowered pleasantness ratings in SABP patients, to the contrary, the SABP group showed highest pleasantness ratings for the CS+ across all experimental phases (suppl. Fig. 26). In contrast to that, arousal levels were in almost all phases higher for the CS+ than for the CS- (suppl. Fig. 25), indicating that in general CS+ trials led to higher arousal levels in the SABP sample. These findings could reflect impairment in appetitive learning on a neuronal level, which was not affecting the perception of pleasantness in the SABP stage.

In contrast to HC, CBP patients also elicited stronger activation of the pCC during appetitive conditioning (Fig. 11B). Activation of the pCC could indicate a higher cognitive load in CBP patients during appetitive learning. The involvement of a cognitive control region such as the pCC (Kanske 2012; Leech et al. 2011) during appetitive learning could reflect a negative control mechanism biasing the appetitive CS+ as more negative and is thus further in line with the reduced NAC activation. This is also reflected in findings, showing that the pCC was activated by painful stimuli in an experiment in which both appetitive and aversive stimuli were used (Rolls 2003). A study with fibromyalgia patients showed that the pCC encoded pain catastrophizing in those patients (Lee et al. 2018). The authors proposed that this activation could reflect ongoing catastrophizing-associated activity while processing pain-related signals. Considering also the lower pleasantness ratings for the CS+ un. throughout the experiment (suppl. Fig. 26) in combination with higher arousal levels (suppl. Fig. 25) support this notion further. Similar findings were reported in fibromyalgia patients, who validated slow brush

movements as less pleasant when compared to HC (Boehme et al. 2020). These findings could indicate that the PT is perceived as less likeable, leading to a weaker modulation of pain by positive affect in patients with CBP (Kamping et al. 2013; Rainville et al. 1997). This is further supported by changes in sensory perception in both pain samples which will be discussed in the next section.

In my study, both pain patient groups indicated a shift towards pain-related brain areas and a decrease in reward-related brain regions in contrast to HC during appetitive learning. This is in line with my hypothesis that appetitive learning is impaired in different stages of back pain. Appetitive learning in SABP and CBP patients was affected differently in contrast to HC. Furthermore, my data indicate that sensory processing is shifted towards pain in situations where subjects should feel pleasantness (see next section). This could lead to subjects losing the pain alleviating features of touch (Williams and Rhudy 2012). Research on fibromyalgia patients already demonstrated that a deficient modulation of pain by positive affect resulted in patients lacking pain reduction during positive picture viewing in contrast to HC (Kamping et al. 2013; Rhudy et al. 2013). In relation to CBP, maladaptive affective modulation of external stimuli could have similar consequences as seen in fibromyalgia patients. Contrary to my expectations, I could not identify significant differences in the used ROIs in the comparison between SABP and CBP patients. This can be explained by the fact that the SABP group is a heterogeneous group consisting of subjects who might still develop CBP and those who might recover.

4.1.1 Impaired processing of appetitive sensory stimuli in patients with SABP and CBP

I hypothesized that already the processing of affective sensory stimuli is altered in different stages of back pain, driving maladaptive learning mechanisms, as was discussed earlier (section 4.1). As a matter of fact, I found changes in the processing of the PT stimulus in the CBP and SABP patients. SABP patients showed a negative correlation between initial US valence ratings and learning-related brain responses in the Hipp, Amy and the IC (Tab. 11). Reflecting the fact that subjects who rated the US stimulation as less pleasant were the ones showing strongest activation in learning-related brain areas (Büchel et al. 1998; Sehlmeyer et al. 2009; Seymour et al. 2005). The Amy and the Hipp were reported to be important in learning and memory processes (Cacciaglia et al. 2015; Chase et al. 2015; Ito, Everitt, and Robbins 2005; Martin-Soelch, Linthicum, and Ernst 2007). This could indicate that learning was more demanding for the SABP sample. The IC was shown to be involved in subjects awareness of well-being, emotional awareness (Craig 2002) and processing of stimulus intensities (Case et al. 2016). Furthermore, IC activation was seen in several studies using appetitive reinforcer during conditioning, such as odours, tastes and touch (Francis et al. 1999; Gottfried, O'Doherty, and Dolan 2002; Olausson et al. 2002), but also in pain and PT (McGlone et al. 2012; Rolls 2003). A study with patients with right IC lesions could show that those patients showed lower pleasantness ratings than controls and that besides intact C-tactile afferents, the lesion in the right IC caused deficits in the

perception of affective touch (Kirsch et al. 2020). Investigations in fibromyalgia patients showed decreased IC activation when positive pictures were shown alongside a painful stimulation. Those subjects revealed impaired modulation of pain by positive affect in contrast to a group of HC, who reported less pain in those trials (Kamping et al. 2013). The PT, which was used as an appetitive US in the experiment, was designed to have stimulus properties which define a stimulus as pleasant, such as stimulation velocities and the texture of the PT (Essick et al. 2010; Löken et al. 2009; Nees et al. 2018). Stimulation site was the left lower arm. Hairy skin was reported to contain C-tactile afferents which are tuned to process affective aspects of touch (Löken et al. 2009; Pawling et al. 2017; Taneja et al. 2019; Triscoli et al. 2017). Slow, light touch was shown to activate the IC (Olausson et al. 2002; McGlone et al. 2012). These findings highlight altered sensory processing in SABP patients, given by the negative correlation between perceived pleasantness ratings and IC activation.

Differences in processing of affective sensory stimuli could have also driven the found changes in appetitive learning mechanisms (section 4.1). To test this, a linear regression model was used within each subject group, where initial brain responses in certain ROIs to the PT stimulation were included as potential predictors of brain responses during appetitive learning. Shared variance in appetitive learning responses was explained by brain activation in areas reported to be involved in processing of stimulus properties or to be critical for decision making and behavioural responses. A significant association between ROI activation during PT stimulation and learning-related brain activation was seen in SABP patients within the mPFC and the OFC which predicted learning in ACQ1, whereas activation in the IC and in the dlPFC predicted appetitive learning in ACQ2 (Tab. 12). ROI activation during PT stimulation in SABP patients established always a positive effect on learning responses, with exception of the mPFC which revealed a negative interrelation. The positive effect of IC and OFC activation on learning, seen in SABP patients, is in line with previous research showing that the OFC and the IC are activated during positive reinforcement (Francis et al. 1999; Kirsch et al. 2003; O'Doherty et al. 2003) and to encode C-tactile afferent PT stimulation (Löken et al. 2009; Rolls 2003). Furthermore, both brain areas were reported to be involved in the valuation of the stimulus properties and shaping the reaction towards the stimulus (Cox, Andrade, and Johnsrude 2005). My data support those findings. Patients who established strong activations in both ROIs were the ones showing stronger BOLD responses during appetitive learning. Furthermore, in SABP patients, also the activation of the dlPFC predicted learning-related brain activation in ACQ2. The dlPFC was reported to be involved in executive control and working memory (Levy and Goldman-Rakic 2000). Higher cognitive control is usually needed in novel situations and to solve difficult tasks. It might be that in the SABP sample more attention was needed during emotional learning at a later time point in the experiment, due to alterations in appetitive processing. Based on the findings of the regression model, it seems likely that, appetitive learning is more demanding for SABP patients, although all subjects acquired contingency awareness (suppl. Fig. 24). Both, SABP and CBP patients, showed a negative relationship between mPFC activation and appetitive learning (Tab. 12). These findings indicate that

weaker mPFC activation resulted in stronger learning-related brain activation. The mPFC is a brain region which is discussed to play an important role in cognitive control, working memory and emotions (Apkarian et al. 2005; Gusnard et al. 2001; Levy and Goldman-Rakic 2000). Weaker activation of the mPFC was seen during attention-demanding tasks (Gusnard et al. 2001). These findings could be an indicator for an affective modulation of learning responses in pain patients. Patients who showed greater impairment in processing of affective stimuli, given by weaker BOLD responses during PT stimulation in the mPFC, were the ones which needed more cognitive control, when presented to a positive sensory stimulation. Thus, these patients performed worse during learning or more precisely exhibited weaker differences in processing between CS+ un. and CS- trials. It might be that those patients benefit less from the PT stimulation, resulting in weaker responses during learning, in the target regions.

Findings in the CBP sample indicate that the processing of the PT stimulation is also impaired. CBP patients did not show additional effects in reward-related brain areas, such as the OFC which was seen in HC and SABP patients (Tab. 12). This indicates that the affective stimulus failed to elicit responses in a brain region known to be important in the processing of positively valenced stimuli (Kamping et al. 2013; O'Doherty et al. 2004) and to play a key role in associative learning and goal-directed learning (O'Doherty et al. 2006). Studies in monkeys with lesions in the OFC showed impairments in learning the association between reward and stimulus (Meunier, Bachevalier, and Mishkin 1997). In humans, OFC lesions led to difficulties in mood identification of others (Hornak, Rolls, and Wade 1996). Further, OFC showed sub-regional specialization for the processing of either appetitive/reward or aversive stimuli/punishment (Gottfried, O'Doherty, and Dolan 2002; O'Doherty et al. 2003). This highlights its importance in associative learning and the maladaptation of the processing of appetitive stimuli in CBP.

My data indicate that the positive affective nature of the used PT stimulation is attenuated in both pain groups, leading to higher arousal levels (suppl. Fig. 25). Processing of the PT seems to be differently affected in the SABP and CBP stage. This could indicate that in the early SABP stage still more processes are active, that will drive the transition to CBP which in turn influence the processing of external appetitive stimuli. Once pain has turned chronic, other brain processes become of greater importance in processing of external stimuli. Connectivity analyses would give better insight in network changes in those subjects.

4.2 Enhanced aversive learning in CBP

I hypothesized that patients with SABP and CBP show enhanced aversive learning responses in contrast to HC. This is supported by increased brain responses in emotion-related brain areas in patients with CBP. Heightened learning-related responses were not corroborated by activation in the Amy, but rather by a differential activation of sub-regions in the IC and a decrease in pain-related brain areas in CBP patients. Brain activation within the IC was comparable between HC and SABP patients, with stronger activation of the left posterior IC and right insular-opercular cortex when compared to CBP patients (Fig. 21). CBP patients showed significant BOLD responses during aversive learning in two clusters in the left IC and one cluster in the right posterior part of the IC (Fig. 21). The IC is a highly connected brain area which was shown to play an important role in integrating sensory-discriminative information, in cognitive-evaluative processes, as well as in affective information of pain characteristics (Peltz et al. 2011; Starr et al. 2009). Due to its cytoarchitecture the IC can be subdivided into the posterior and anterior IC (Kurth et al. 2010). Besides its different cytoarchitecture, processing in those subdivisions were also shown to be distinguishable. It was shown that activity in the posterior IC directly reflects the intensity of a given painful stimulus (Carlsson et al. 2006; Peltz et al. 2011; Seifert and Maihofner 2009; Singer et al. 2004). In addition, it was activated in anticipation of predictable heat stimulation (Carlsson et al. 2006). Processing of affective stimuli was ascribed to be mainly processed in the anterior IC (Büchel et al. 1998; Singer et al. 2004). Receiving a painful stimulation activated the posterior IC, but seeing a loved one being in pain activated the anterior IC in test subjects (Singer et al. 2004). The IC is the only brain area which can elicit pain if it is electrically stimulated (Mazzola et al. 2009, Ostrowsky et al. 2002). These findings highlight the involvement of the IC in pain and its possible influence in chronic pain. Furthermore, activation of the right frontal operculum and the anterior IC was seen in both, HC and SABP patients when compared to CBP patients (Fig. 21A and Fig. 21D). The IC, as well as the frontal operculum were discussed to be important in the cortical representation of pain (Treede, Baumgärtner, and Lenz 2007), reacting with shortest latencies to external painful stimuli (Frot and Mauguiere 2003). Patients with lesions in the opercular-insular cortex suffered from deficits in pain and heat sensations (Garcia-Larrea et al. 2010). A study using different nociceptive inputs, could show that the opercular-insular cortex elicited a distinguishable somatotopic representation of the used painful stimuli and body-site (laser stimulus or pin-prick, hand or foot) in the contralateral hemisphere to the stimulation site (Baumgärtner et al. 2010). These findings are in line with the results of the IC and frontal opercular activation during aversive learning and their sub-regional specificity seen in the three subject samples. The significant responses in the IC and frontal operculum could simply reflect association between the CS+ un. and the aversive US, although no pain stimuli were presented in those trials. This could indicate that learning was already established and that the CS+ un. acquired the same significance as the US, predicting pain. This is further supported by the behavioural data which highlight that all subjects acquired contingency awareness (Fig. 29). Proof of sub-regional specificity

in the IC in pain patients is so far missing. Since the IC is an important node in pain perception and modulation, its involvement in the development of CBP is likely. Heightened posterior IC activation during pain ratings and deactivation during pleasantness ratings, which was coupled to grey matter decreases in the anterior IC was seen in fibromyalgia patients (Boehme et al. 2020). The opposing effect was seen in the tested control group of HC. Baliki and colleagues (2006) showed that IC activation reflected acute thermal pain in CBP patients (Baliki et al. 2006). The authors further showed that activation in the right anterior IC reflected pain persistence in those patients. Grey matter decreases in the right IC and reduced connectivity between IC and other brain areas was observed in SABP patients who developed CBP (Baliki et al. 2012). The authors proposed that the IC contributes to the development of chronic pain (Baliki et al. 2012). My findings indicate that these sub-regional activation patterns seen in the IC during aversive learning could elucidate its involvement in the chronicity process (Fig. 21 A-D). In addition, CBP patients showed lower activation in the pCC, IC and the S1 when compared to HC (Fig. 22). Activation of pain-related brain areas, such as the IC, S1 and the ACC, was seen in anticipation and expectation of pain (Ploghaus et al. 1999; Porro et al. 2002; Villemure and Bushnell 2002). Moreover, the somatosensory cortex (S1 and S2) was also reported to be activated during early anticipatory activation during CS+ processing in studies using visceral pain as US (Gramsch et al. 2014; Kattoor et al. 2013). Both, the pCC and the IC are brain areas, which were characterised by activation during sensorimotor processing and self-relevant sensations (Carlsson et al. 2006; Lee et al. 2018; Vogt 2005). Furthermore, activation in both brain areas was seen during the processing of a potentially threatening stimulus (Berret et al. 2019). Moreover, the pCC was characterised by being involved in cognitive control (Kanske 2012; Leech et al. 2011). A decrease in pain-related brain areas was already reported in CBP (Apkarian et al. 2005), similar to findings in this thesis. Those decreases were reported to reflect reduced sensory processing in those brain areas, accompanied by heightened emotional and cognitive processing in CBP (Apkarian et al. 2005). A decrease in pCC activation was also reported in fibromyalgia patients during painful stimulation (Lee et al. 2018). My findings indicate that CBP patients show alterations in aversive learning mechanisms, given by a decreased activity in pain-related brain areas and enhanced cognitive processing in contrast to the HC sample.

Apart from changes in CBP, there were also differences in SABP patients compared to HC. SABP patients showed stronger activation of the ACC in contrast to HC (Fig. 21E). The ACC has been found to be related to the unpleasantness of a pain stimulus (Rainville et al. 1997). The authors proposed that pain-evoked activity in the ACC would shape the behavioural and emotional reaction towards pain (Rainville et al. 1997). Moreover, it is discussed to play a crucial role in early associative learning (Carlsson et al. 2006). This is further supported by findings in the behavioural data which revealed that SABP patients showed the highest arousal and unpleasantness ratings (suppl. Figures 30-31). Findings in rats support this further. The ACC was shown to be important in avoidance learning and to be mandatory to establish aversive learning, driven by the aversive stimulus (Johansen and Fields 2004).

ACC activity in the SABP sample could therefore simply reflect ongoing associative learning processes during the acquisition of the aversive CS+ un. and planning of behavioural responses. Higher activity in the ACC could indicate that aversive learning is enhanced in SABP patients compared to the HC.

4.2.2 Impaired processing of aversive sensory stimuli in patients with SABP and CBP

I hypothesized that already the processing of the aversive sensory stimuli is altered in different stages of back pain, driving maladaptive learning mechanisms (section 4.2). My findings indicate that the initial responses during painful stimulation heightened attention, pain perception and arousal levels during aversive learning in HC and CBP patients, but not in SABP patients. HC showed significant negative correlations between initial US valence ratings in HAB and aversive learning responses in the ACC, dlPFC and S2 in ACQ1 and in the ACC, NAC, Hipp, Amy, OFC and the S2 in ACQ2 (Tab. 21). The more subjects rated the aversive US as unpleasant (= lower valence), the stronger the brain activation during aversive learning was. These findings are in line with my expectations to see strong activation in brain regions which were already reported to be involved in the acquisition of aversive delay conditioning (ACC, Amy), fear learning (ACC, Amy Hipp, NAC, S2) (Phelps et al. 2001; Sehlmeyer et al. 2009) and prediction error processes and expectancy of the occurring painful stimulation (NAC) (Baliki et al. 2010; Büchel et al. 1998; Phelps et al. 2001; Seymour et al. 2004). Uncertainty about the upcoming US presentation was reported to be reflected by activation in the dlPFC and the OFC in trials with unpredictable outcome (Carlsson et al. 2006; Phelps et al. 2001; Seymour et al. 2005). The correlational data indicate that the HC sample was able to establish the expected behavioural and neuronal responses during aversive learning. I would have expected to see a negative correlation between the mPFC and valence ratings in the CBP sample, as proposed in neuroimaging studies which reported a shift towards brain areas involved in emotional processing in CBP patients (Baliki et al. 2006). These differences could be due the fact that I tested emotional learning responses, whereas Baliki and colleagues (2006) focused on brain activation patterns during painful stimulation in HC and CBP patients. A review article comparing neuroimaging studies using conditioning data highlighted the fact that due to differences in experimental designs (reinforcement rates, instructions given to the subject, stimulation site and used stimuli), heterogeneous results are often reported (Sehlmeyer et al. 2009).

I was interested to test, whether pain patients show heightened responses during aversive stimulation, influencing aversive learning. Brain areas which are critical for the processing of nociceptive and non-nociceptive stimuli and for cognitive control were activated by the aversive US and predicted learning-related BOLD responses. My findings indicate that the initial responses during painful stimulation heightened attention, pain perception and arousal levels during aversive learning in HC and CBP patients, but not in SABP patients. Processing of the painful stimulation seemed to affect

learning responses in SABP and CBP patients differently. A significant association between ROI activation during painful stimulation and learning-related brain activation was found in HC in the mPFC and the S2 in ACQ1, whereas activation in the IC and in the NAC predicted aversive learning in ACQ2 (Tab. 22). ROI activation during aversive stimulation established a negative impact on learning in ACQ1 and a positive effect on learning during ACQ2. Stronger activation of the mPFC and S2 during initial painful stimulation in the HAB reflected that HC subjects showed weaker distinguishable brain responses for the CS+ un. and CS- trials, resulting in weaker aversive learning-related brain activation in the used learning contrast. This could reflect ongoing cognitive load during ACQ1, where subjects still needed to establish contingency awareness given by the activation in the mPFC. As aforementioned, the mPFC was discussed to play an important role in cognitive control, working memory (Apkarian et al. 2005; Levy and Goldman-Rakic 2000). In addition, it was activated during the threat signal (CS+) in an aversive conditioning paradigm, but not for the safety signal (CS-) (Pohlack et al. 2012). The entire subject sample was able to assess the contingency awareness (suppl. Fig. 29). Thereby, stronger activation in the mPFC during the initial painful stimulation could reflect that those subjects tried to prepare themselves for the painful stimulation (Wiech, Ploner, and Tracey 2008). The negative association between S2 activation during initial painful stimulation and aversive learning responses in HC can be similarly explained, simply reflecting early anticipatory activation during CS+ processing. Both, IC and NAC activation statistically predicted learning responses in HC in ACQ2. Higher prestimulus activity in the IC was shown to lead to increased pain perceptions in HC (Ploner et al. 2010). These findings are in line with Langs' priming hypothesis (Lang 1995) which stated that the emotional state of the organism will define its perception of external affective stimuli. NAC activity during initial painful stimulation predicted learning responses during ACQ2 in HC. The NAC was associated to mediate cue-outcome associations and to reflect prediction error processes during learning (Jensen et al. 2007). In ACQ2 contingency awareness was established (suppl. Fig. 29), but subjects were not informed about reinforcement rates and the trial order was pseudo-randomized. Therefore, subjects never knew when coupling between CS+ presentation and US occurred or was omitted. The positive relationship between NAC and aversive learning is in line with my expectations, reflecting ongoing learning.

S1 activation during painful stimulation predicted aversive learning in CBP patients in ACQ1, whereas the ACC and the NAC predicted learning in ACQ2 (Tab. 22). The S1 is, as aforementioned, active during early anticipation of painful stimulation (Gramsch et al. 2014; Kattoor et al. 2013). It is further characterised to be activated by attention shifted towards the used stimulation (Bushnell et al. 1999). Initial painful stimulation focussed the attention of the CBP patients on the painful stimulation, which resulted in stronger learning-related brain activation. This could indicate that those subjects showed higher attention towards ongoing processes during conditioning. NAC activation in the CBP group could reflect, similar to HC, prediction error processing, further supporting that CBP patients were actively following the experiment. Both, NAC and ACC, were reported to be activated during error

detection and expectancy violations (Dunsmoor and LaBar 2012). This is again in line with my expectations that subjects needed to follow the experiment, since they were not aware about reinforcement rates between CS+ and US presentation. ACC activation was shown to correlate with the unpleasantness of the used pain stimulus but not with its intensity (Rainville et al. 1997). The authors proposed that pain-evoked activity in the ACC will shape the behavioural and emotional reaction towards pain (Rainville et al. 1997). This was further supported by Ploner and colleagues (2010) who could show that prestimulus activation in the ACC affected perceived pain intensity in their HC sample (Ploner et al. 2010). The painful stimulation during HAB affected brain responses during aversive learning in CBP patients. The painful stimulation possibly shaped the emotional state of the CBP patients, whereby the perception of how painful CS+ trials were perceived was affected. Similar to the IC effect on learning responses in HC, the initial ACC activation in CBP patients could have heightened pain perception in those subjects, reflected by higher arousal and lower valence ratings in CBP patients (suppl. Figures 30-31).

OFC activity during painful stimulation established a statistically negative correlation with aversive learning responses in SABP patients during ACQ2 (Tab. 22). The OFC is characterised to encode outcome expectancies and to facilitate associative learning (Martin-Soelch, Linthicum, and Ernst 2007; Gottfried, O'Doherty, and Dolan 2002; Seymour et al. 2005). The negative interrelation seen in the SABP sample could reflect that learning was already established in ACQ2, therefore the OFC did not predict learning in ACQ2 (suppl. Fig. 29), because subjects already acquired outcome expectancies between CS+ and US trials. A study investigating patients with irritable bowel syndrome in an aversive conditioning paradigm with rectal distension as aversive US, could show that patients exhibited stronger activation of the OFC during CS- processing in contrast to the HC. Tested in this thesis, was always the learning contrast between CS+ un. > CS- trials. Therefore, the negative interrelation seen between OFC activity during initial US presentation and learning responses could reflect that the OFC would have had better predictions for the safety signal (CS-) rather than for the learning contrast.

My data indicate that the initial pain stimulation shaped subjects' learning behaviour by heightening attention and perceived unpleasantness in CS+ un. trials which enhanced learning-related BOLD responses in HC and in CBP patients, but not in the SABP sample. Patients with SABP only established a negative interrelation between OFC activity and later learning responses. This indicates that in the SABP patients different processes are active than in HC and CBP patients, with a key role of the OFC in processing of sensory stimuli in the SABP stage.

4.3 Limitations and Outlook

Clearly, there are several limitations in this thesis. First, I have to assume that the group of SABP patients is heterogeneous, consisting of subjects who will recover and those that develop CBP. To get a better understanding of early stages of back pain, I would need to subdivide the SABP sample into persisting pain and recovery from pain to identify which mechanisms are really ascendant of CBP. Longitudinal data would help to identify whether maladaptive emotional learning is a predictor for the development of CBP. One drawback of my approach is the used masks for the ROI analyses. The masks were large and always covering both hemispheres. More fine graduated masks would help to elucidate findings in appetitive and aversive learning data and would help to increase the power in my data, particularly in the aversive conditioning data. A conjunction analysis of within group responses, especially in the IC, could reveal whether the sub-regional specificity is also seen on the group level and if subject groups can be subdivided based on IC activation. Further, involvement of the IC in appetitive learning would be interesting to test and to compare whether activation patterns within each group can be differentiated between appetitive and aversive learning. Moreover, I did not consider any structural or functional changes in my thesis. Both were reported to predict the development of CBP (Baliki et al. 2006; Makary et al. 2020; Vachon-Presseau, Centeno, et al. 2016). Previous research highlighted the impact of maladaptive extinction processes in CBP (Schneider, Palomba, and Flor 2004). Extinction analyses would clarify whether extinction processes are already impaired in the SABP sample. Moreover, the used electrical stimulation in the aversive learning experiment was subjectively adjusted to subject's pain thresholds. Therefore, US intensities could have been too weak to elicit fear in the study samples. Patients with higher pain-related fear were shown to over-predict novel pain and to stop painful leg raises earlier in an experimental set-up (McCracken et al. 1993). Therefore, excluding subjects with missing BOLD responses during US stimulations could further enhance statistical power in my data. Pain levels before conditioning were shown to impact CRs in patients with CBP and tension headache patients (Klinger et al. 2010). Pain levels were assessed on each measurement day and should be considered in the analyses. Lastly, my data would be suitable to test the proposed pain signature of Wager and colleagues (Wager et al. 2013) and to compare it to the seen maladaptive changes in both pain groups. Despite the reported shortcomings in this thesis, my data reveal that emotional learning mechanisms seem to be altered in different stages of back pain, with a differential impact of dysfunctional sensory processing on those mechanisms. The chosen SABP sample helped to elucidate ongoing changes in early back pain stages. SABP patients seem to be a suitable group to investigate the transitional phase in the chronicity process. Longitudinal data would help to disentangle the reported findings and to highlight which processes play a key role in the development of CBP.

4.4 Summary and Conclusions

My data show that emotional learning processes are altered depending on the pain symptom stage. This indicates that those processes might be critical in the persistence of pain symptoms, ultimately leading to pain chronicity. Pain patients showed a shift away from striatal and limbic brain areas towards more pain-related brain areas during appetitive learning, with reduced activation in reward-related brain areas. This might be partially driven by the observed alterations in the perception of the appetitive sensory stimulus. These changes are indicated by an increase in activity in pain-related brain areas which could have induced allodynia, rather than still triggering an appetitive perception in chronic pain. This is also reflected in the finding that responses to the appetitive sensory stimulus in the OFC, a region often reported during the processing of pleasant stimuli, predicted learning only in SABP patients and HC, but not in patients with CBP. It is therefore likely that CBP patients benefit less from positive external stimuli and rather focus on pain. My findings highlight the importance of the emotional appetitive learning in different back pain stages. A specific role of appetitive learning in the development and maintenance of CBP is likely.

My data also reveal an important role of the limbic system in aversive learning, with a significant influence of the IC and the ACC. Aversive learning mechanisms seem to be differently affected in SABP and CBP, reflected by sub-regional specific activation patterns seen in the IC. The ACC revealed a strong involvement in both pain samples during aversive learning, with a distinguishable influence in the SABP and CBP stage. My findings indicate that the initial pain stimulation shaped subjects' learning behaviour by heightening attention and perceived unpleasantness during aversive learning in HC and CBP patients, but not in SABP patients. CBP patients showed a decrease in pain-related brain areas during learning, whereas the OFC revealed an inverse association between initial pain processing and aversive learning-related responses in SABP patients. Emotional learning responses in SABP patients seemed to be driven by early responses in the OFC, irrespective of the used affective stimulus. It could be a key brain area affected by maladaptive changes, influencing sensory processing and thereby paving the path for chronicity. My findings suggest that different brain processes are active during early and late stages of CBP, with a crucial involvement of the IC and the ACC in aversive learning.

SABP patients are in a critical transition period in which they either develop chronic pain or show resilience. Here, emotional learning could be a key mechanism to determine the possible development of CBP. Altered processing of appetitive stimuli might represent an important modulating factor in this context. My data suggest that emotional learning may be an important mediator shifting acute to chronic pain. Treatment interventions should reverse aversive and enhance appetitive pain-related memories.

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6. Supplement

6.1 Behavioural and physiological assessments during appetitive conditioning

6.1.1 Contingency Awareness Ratings

Contingency ratings during appetitive conditioning across all groups showed a significant effect of stimulus (CS+ versus CS-) in all phases where the US was presented (HAB: $F(2,230) = 17.03$; $p < .001$ ACQ1: $F(2,230) = 626.37$, $p < .001$, ACQ2: $F(2, 230) = 366.21$, $p < .001$, EXT: $F(2,230) = 3.06$, $p = .082$), but there was no significant effect of group or the interaction between stimulus*group. Along this line, ratings within each group were significantly different between the CS+ and the CS- in ACQ1 (HC $t(36) = -14.51$, $p < .001$, SABP: $t(46) = -17.19$, $p < .001$, CBP $t(33) = -9.57$, $p < .001$) and ACQ2 (HC: $t(36) = -12.71$, $p < .001$, SABP: $t(46) = -16.80$, $p < .001$, CBP: $t(33) = -10.39$, $p < .001$) (Tab. 23). As expected contingency awareness during EXT did not lead to any significant results, since the US presentation was stopped during this learning phase. All subject samples learned the coupling between the US and CS+ during both acquisitions and that the CS- was never paired to the US (Fig. 24 and Tab. 23).

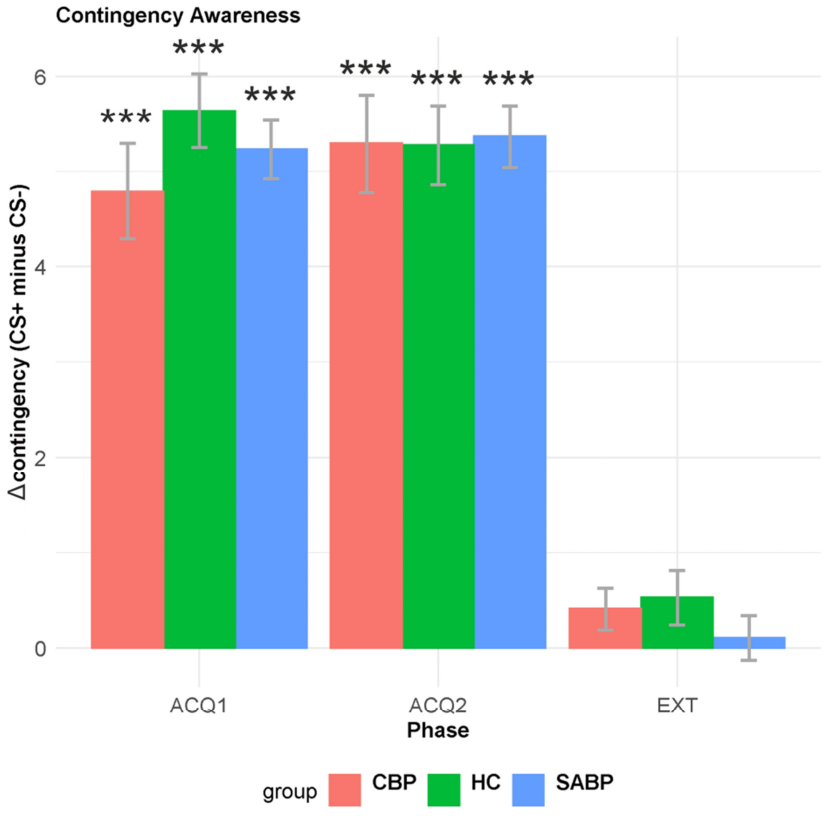

Figure 24: Contingency awareness (CS+ un. minus CS-). Subjective ratings of the contingency awareness between the CS+ and the appetitive US in HC (green), SABP (blue) and CBP patients (red). Delta CS was calculated using the mean CS+ ratings minus the mean CS- ratings within each group, depicted on the y-axis. X-axis represents ratings for each phase and group, respectively. Error bars depict the standard error of the

mean (SEM). Asterisks show significant differences in CS+ versus the CS- ratings within one group (post-hoc t-test, corrected for multiple comparisons with the FDR) with significance thresholds *p < .05, **p < .01, ***p < .001. All groups learned the coupling between CS+ and US during ACQ1 and ACQ2. There was no significant difference between CS+ and the CS- trials, during EXT in all subject groups. Group comparisons did not show any significant differences between the samples (Tab. 24).

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	2.35 *	-14.51 ***	-12.71 ***	-1.83	36
	p	0.04	3.38^{-16}	1.69^{-14}	0.11	
SABP	**t**	2.40	-17.19 ***	-16.80 ***	-0.45	46
	p	0.04	3.55^{-21}	2.52^{-20}	0.65	
CBP	**t**	1.36	-9.57 ***	-10.39 ***	-1.84	33
	p	0.18	4.81^{-11}	6.09^{-12}	0.11	

Table 23: Statistical test of within group comparisons of contingency awareness. A paired t-test corrected for multiple comparisons (FDR), was used to calculate within group differences, for the evaluation of the CS+ and CS- (* p < .05, **p < .01, ***p < .001) in each group, respectively. During both acquisitions, ratings for the CS+, within one group, were significantly different from the ratings for the CS-, in all three subject groups. All subjects learned the contingency between CS+ and the US in both acquisitions. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance threshold.

Direct group comparisons between two group means (post-hoc t-test) in contingency awareness ratings did not lead to any significant group differences (Fig. 24 and Tab. 24).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	-0.18	0.82	-0.12	1.13
	p	0.86	0.46	0.98	0.52
	df	75.65	70.84	69.82	72.36
HC/ CBP	**t**	-0.69	1.33	-0.02	0.32
	p	0.86	0.46	0.98	0.75
	df	67.88	63.12	64.55	64.88
SABP/ CBP	**t**	0.54	-0.75	-0.08	0.94
	p	0.86	0.46	0.98	0.52
	df	72.12	56.37	57.47	77.85

Table 24: Statistical comparison of contingency awareness group means. An unpaired t-test (FDR corrected) was used to compare Delta CS (CS+ minus CS-) results comparing, always two group means to each other. There were no significant group differences in contingency awareness. t = t-statistics, p = p-value, df = degrees of freedom.

6.1.2 Arousal Ratings

Arousal ratings for the CS+ versus the CS- showed a significant effect of stimulus only during the HAB and the EXT (HAB: $F(2,230) = 18.15$; $p < .001$, $F(2,230) = 5.16$, $p = .020$), but there was no significant effect of group or for the interaction between stimulus*group. Along this line, arousal levels within CBP patients were significantly different for CS+ and CS- across all phases (ACQ1: $t(33) = -3.27$, $p = .008$, ACQ2: $t(33) = -2.68$, $p = 0.034$) (Tab. 25). SABP patients rated the CS+ as significantly more arousing than the CS- in the HAB and EXT (HAB: $t(46) = 3.98$, $p < .001$, EXT: $t(46) = -2.77$, $p = .24$), but not during both acquisitions (Fig. 25 and Tab. 25). SABP patients showed higher arousal levels after the first exposure to the used stimuli (HAB) and after the US presentation was stopped (EXT). HC showed significant different arousal levels for the CS+ and the CS- during HAB (Fig. 25 and Tab. 25). Both pain patient groups showed higher arousal levels in contrast to HC (not significant = n.s.). Group comparisons did not yield any significant differences between groups (Fig. 25 and Tab. 26).

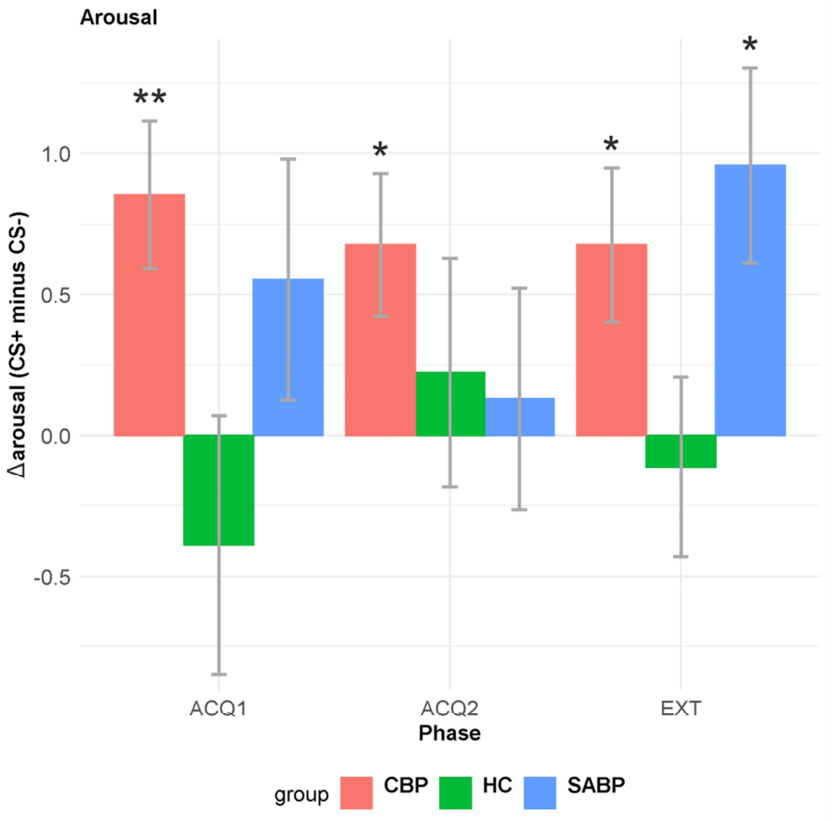

Figure 25: Arousal levels (CS+ minus CS-). Subjective arousal ratings (Delta CS ± SEM) in HC (green), SABP (blue) and CBP patients (red) depicted on the y-axis, across different experimental phases (x-axis). Asterisks show significant differences in CS+ versus the CS- ratings, within one group (post-hoc t-test, FDR corrected) with significance thresholds *p < .05, **p < .01. CBP patients rated the CS+ as significantly more arousing than the CS-, in all phases of the experiment. SABP patients showed a significant different arousal level for the CS+

versus the CS- after the EXT, but not during acquisitions. Group comparisons did not show any significant difference between subject samples (Tab. 26).

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	-2.90 **	0.84	-0.55	0.34	36
	p	6.40^{-3}	0.40	0.74	0.73	
SABP	**t**	3.98 **	-1.29	-0.33	-2.77	46
	p	2.14^{-3}	0.30	0.74	0.24	
CBP	**t**	3.49 ***	-3.27 **	-2.68 *	-2.48 *	33
	p	7.26^{-4}	7.53^{-3}	0.03	0.03	

Table 25: Statistical test of within group comparisons of arousal ratings. A paired t-test (FDR corrected) was used to calculate within group differences for the evaluation of the CS+ versus the CS- (*p < .05, **p < .01, ***p < .001) in each group, respectively. During both acquisitions ratings for the CS+ were significantly different from the CS-, in patients with CBP. In SABP patients arousal levels for CS+ and CS- were only significant different in the HAB and in the EXT. HC showed significant arousal levels during the HAB, but not in the other learning phases. Both pain patient groups showed higher arousal levels than HC, across the experiment (n.s.). t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	0.31	-1.50	0.17	-2.28
	p	0.76	0.21	0.87	0.08
	df	73.36	77.49	78.16	80.76
HC/ CBP	**t**	-0.83	-2.35	-0.94	-1.88
	p	0.61	0.67	0.52	0.09
	df	54.65	55.07	58.03	66.98
SABP/ CBP	**t**	1.36	0.60	1.19	-0.64
	p	0.54	0.55	0.52	0.53
	df	75.54	72.65	74.74	78.63

Table 26: Statistical comparison of arousal group means. An unpaired t-test (FDR corrected) was used to compare Delta CS (CS+ minus CS-) arousal levels, comparing always two group means to each other. There were no significant group differences in Delta CS arousal levels between subject samples across all experimental phases. t = t-statistics, p = p-value, df = degrees of freedom.

6.1.3 Valence Ratings

The analyses of the perceived pleasantness ratings during appetitive conditioning, across all groups and phases, showed a significant effect of stimulus in all phases (HAB: $F(2,230) = 10.11$; $p = .002$, ACQ1: $F(2,230) = 54.68$; $p < .001$, ACQ2: $F(2, 230) = 37.48$, $p < .001$, EXT: $F(2,230) = 16.19$; $p < .001$), but no significant effect of group, or the interaction between group*stimulus. Within group comparisons for the CS+ versus CS- ratings resulted in similar findings as in the ANOVA with significant different valence ratings for both stimuli in all groups and across all phases (ACQ1: HC: $t(36) = -5.02$, $p < .001$, SABP: $t(46) = -6.76$, $p < .001$, CBP: $t(33) = -3.53$, $p < .001$, ACQ2: HC: $t(36) = -4.12$, $p < .001$, SABP: $t(46) = -5.58$, $p < .001$, CBP: $t(33) = -4.40$, $p < .001$) (Fig. 26 and Tab. 27). Valence ratings of the CBP patients were lower for the appetitive CS+ versus CS-, across all phases of the experiment in contrast to HC and patients with SABP (Fig. 26 and Tab. 27).

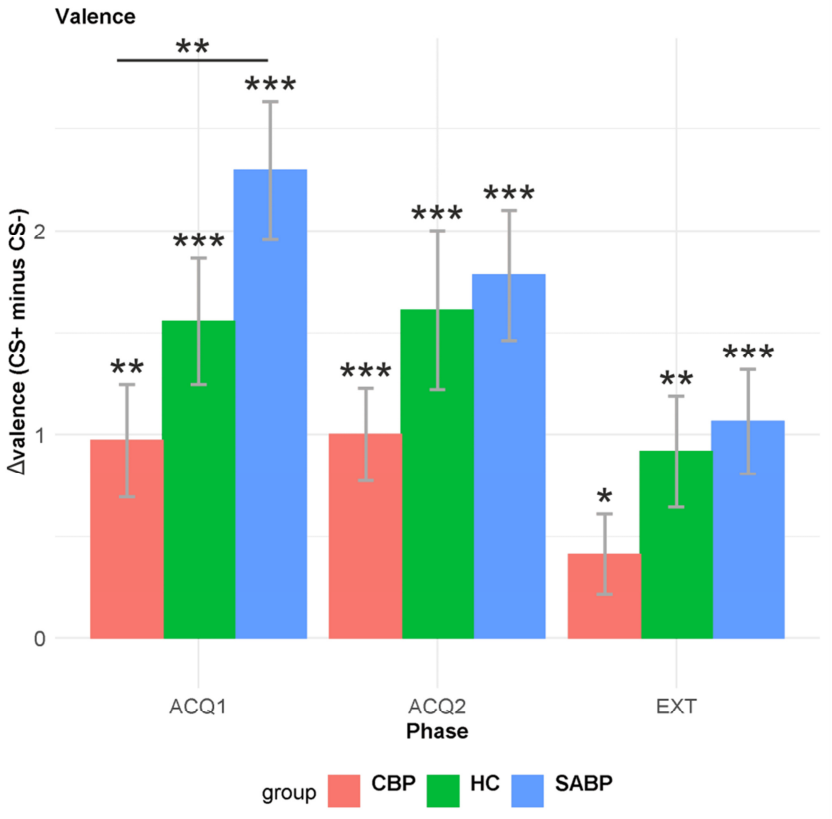

Figure 26: **Valence ratings (CS+ minus CS-).** Subjective ratings of valence evaluation (Delta CS ± SEM) in HC (green), SABP (blue) and CBP patients (red) depicted on the y-axis, across different experimental phases (x-axis). Asterisks show significant differences in CS+ versus the CS- ratings within one group (post-hoc t-test, FDR corrected) and significant group differences (unpaired post-hoc t-test) are depicted with a straight line and asterisks with significance thresholds (*p < .05, **p < .01, ***p < .001). HC and pain patients with SABP and CBP showed significant different valence levels, for the CS+ versus the CS-, across the experiment. CBP patients evaluated the contrast between CS+ minus CS- as less pleasant in all phases in contrast to both, HC and SABP patients (n.s.). In the ACQ1 CBP patients showed significantly lower valence ratings in contrast to SABP patients (Tab. 27).

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	-2.25 *	-5.02 ***	-4.12 ***	-3.38 **	36
	p	0.05	2.25^{-5}	2.19^{-4}	2.70^{-3}	
SABP	**t**	-2.83 *	-6.76 ***	-5.78 ***	-4.14 ***	46
	p	0.02	1.26^{-3}	1.85^{-6}	4.41^{-3}	
CBP	**t**	-1.19	-3.53 ***	-4.40 ***	-2.07 *	33
	p	0.24	6.27^{-8}	1.61^{-4}	0.05	

Table 27: Statistical test of within group comparisons of valence ratings. A paired t-test (FDR corrected) was calculated within each group, respectively, for the evaluation of the CS+ and CS- (*p < .05, **p < .001, ***p < .001). Valence ratings for the CS+ were significantly different from the CS- in all groups. CBP patients showed overall the lowest valence ratings which were only in the ACQ1 significantly different from SABP patients, but not in any other experimental phase, or in contrast to HC. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

Delta CS group comparisons resulted in significant different valence levels between patients with CBP and with SABP during ACQ1, in the ANOVA and in the unpaired t-test ($F(2,114) = 4.45$, $p = .014$ and $t(78.85) = -3.03$, $p < .01$). There were no significant differences between HC and both pain patient groups (Fig. 26 and Tab. 28).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	-0.33	-1.61	-0.43	-0.39
	p	0.74	0.16	0.67	0.69
	df	78.32	80.85	72.30	78.13
HC/ CBP	**t**	1.11	1.41	1.35	1.50
	p	0.41	0.16	0.27	0.21
	df	63.44	67.47	55.90	63.28
SABP/ CBP	**t**	-1.52	-3.03 **	-2.13	-2.01
	p	0.40	9.78^{-3}	0.11	0.14
	df	78.36	78.85	77.10	78.38

Table 28: Statistical comparison of valence group means. An unpaired t-test (FDR corrected) was used to compare Delta CS arousal levels (*p < .05, **p < .01) between subject samples. There was a significant group difference in Delta CS valence levels between both pain patient groups in ACQ1, but not in any other experimental phase. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

6.1.4 Skin conductance responses

A significant effect of group was found in SCRs between CBP patients and HC across all phases (HAB: $F(2,164) = 12.69$, $p = .006$, ACQ1: $F(2,164) = 12.39$, $p < .001$, ACQ2: $F(2,164) = 9.94$, $p < .001$, EXT: $F(2,164) = 10.54$, $p = .003$) and between SABP patients and HC (HAB: $F(2,164) = 12.69$, $p < .001$, ACQ1: $F(2,164) = 12.39$, $p < .001$, ACQ2: $F(2,164) = 9.94$, $p = .0003$, EXT: $F(2,164) = 10.54$, $p < .001$), but not between the patient samples (Fig. 27). There was neither significant effect of stimulus, nor for the interaction of stimulus*group. Statistical tests for within group differences in SCR between CS+ and CS- trials did not yield any significant results (Fig. 27 and Tab. 29). SCR for CS+ and the CS- showed a similar course within patients with CBP and HC, the latter with highest SCR levels and the most distinguishable SCR for the CS+ and CS- (n.s.). SCR for CS+ and CS- trials within patients with SABP were less distinguishable than in the other two subject samples (Fig. 27 and Tab. 29).

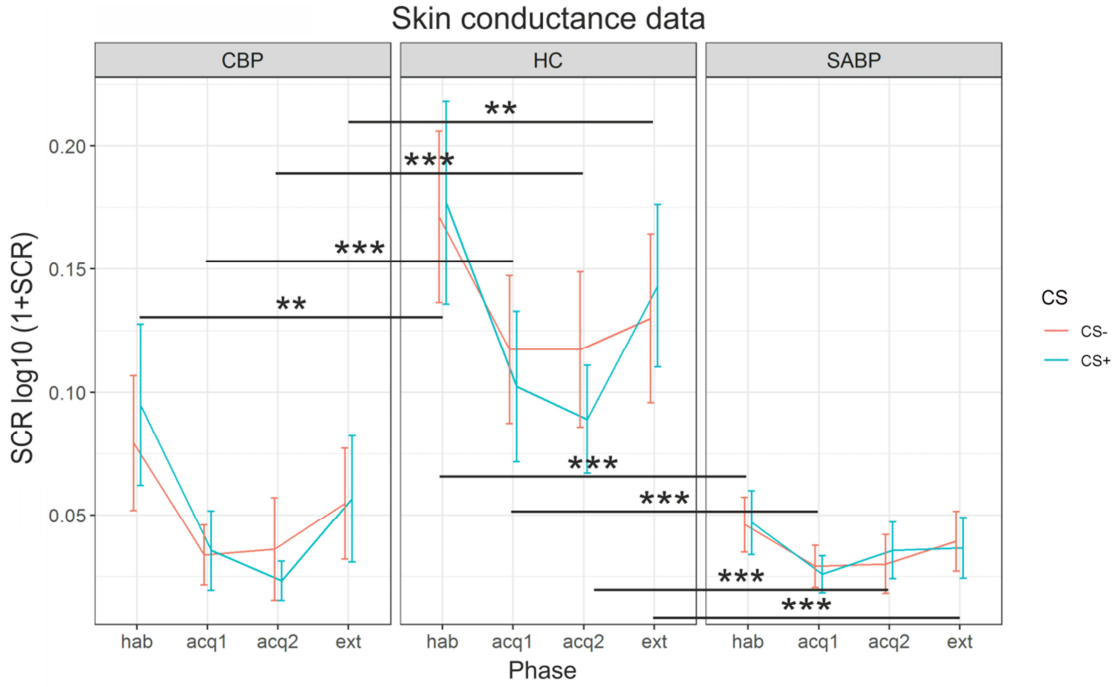

Figure 27: Skin conductance responses (CS+ versus CS-) during appetitive conditioning. Log transformed (log10) skin conductance response (SCR) data (µS) for CS+ responses (blue) and CS- responses (red), depicted on the y-axis, across all four experimental phases (x-axis), for each subject sample separately (subplots). Error bars Delta SCR data ± SEM. Significant effects of group (ANOVA) were marked with straight lines highlighting between which groups and in which phase of the experiment. Asterisks depict significance threshold (*p < .05, **p < .01, ***p < .001). SCRs across the experimental phases showed a similar course within each of the three subject samples, with different levels of SCRs which became only significant in the ANOVA between both pain patient groups in contrast to HC. This significant difference was seen across all phases of the experiment where the US was presented (HAB, ACQ1, ACQ2). These significant group effects did not yield any significant results in the post-hoc t-tests. HC showed the biggest difference in SCRs for the CS+ and the CS- (n.s.) and SABP patients showed the lowest difference in SCRs for both stimuli. Within group comparisons of the SCRs, for the CS+ and the CS-, did not yield any significant results in the three subject samples.

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	-0.35	0.96	1.42	-0.62	24
	p	0.90	0.71	0.36	0.80	
SABP	**t**	-0.13	0.72	-0.93	0.68	34
	p	0.90	0.71	0.36	0.80	
CBP	**t**	-1.71	-0.23	0.94	-0.26	23
	p	0.30	0.82	0.36	0.80	

Table 29: Statistical test of within group comparison of SCRs for the CS+ and the CS-. A paired t-test (FDR corrected) was calculated within each group, respectively, for the log10 transformed SCR data of the CS+ and CS-. There were no significant differences for SCRs for CS+ and CS- trials across, all subject samples and phases of the experiment. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

In a second step, group comparisons were calculated for the Delta CS SCRs. There were no significant group differences in the ANOVA in Delta CS SCRs, therefore also no significant differences could be found in the unpaired t-tests (Tab. 30). Delta CS results in HC and in SABP patients showed almost the opposite progression throughout the experiment, both ranging around 0 µS (Fig. 28). CBP patients showed a strong decrease in SCRs from HAB to ACQ2 and ascending during the EXT. All three subject groups showed an increase in SCRs during EXT, with HC showing highest SCRs and CBP patients lowest SCRs (Fig. 28 and Tab. 30).

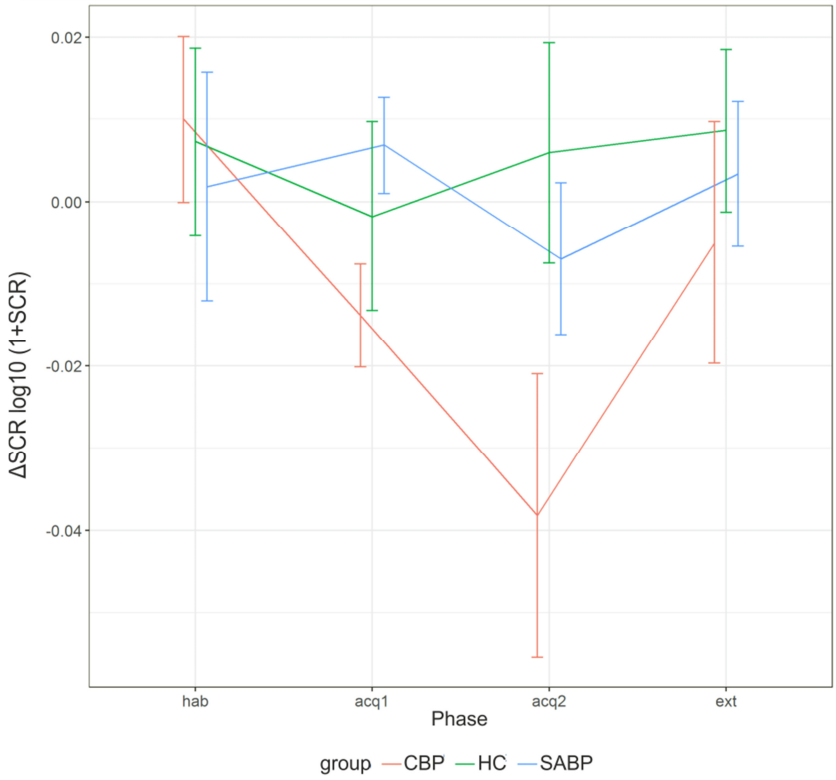

Figure 28: Skin conductance responses (Delta CS) during appetitive conditioning. Log transformed (log10) SCRs (y-axis) for HC (green), SABP (blue) and CBP patients (red), respectively, across all experimental phases (x-axis). Error bars Delta CS ± SEM. Overall group comparison did not yield any significant differences between subject samples (Tab. 30).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	0.30	-0.67	0.79	0.40
	p	0.86	0.50	0.43	0.69
	df	57.96	36.46	45.06	53.60
HC/ CBP	**t**	-0.18	0.92	2.02	0.77
	p	0.86	0.50	0.15	0.69
	df	56.17	37.17	43.83	40.49
SABP/ CBP	**t**	0.48	-2.41	-1.60	-0.49
	p	0.86	0.06	0.18	0.69
	df	56.17	53.14	36.08	39.14

Table 30: Statistical group comparisons of SCRs. An unpaired t-test (FDR corrected) was calculated between all subject samples. There were no significant group differences. t = t-statistics, p = p-value, df = degrees of freedom.

6.2 Behavioural and physiological assessments during aversive conditioning

6.2.1 Contingency Awareness Ratings

Contingency ratings during aversive conditioning across all groups showed a significant effect of stimulus, in all phases where the aversive US was presented in addition to the CSs (HAB: $F(2,236) = 15.05$, $p < .001$, ACQ1: $F(2,236) = 482.51$, $p < .001$, ACQ2: $F(2,236) = 519.58$, $p < .001$, EXT: $F(2,236) = 1.27$, $p = .26$). There was no significant effect of group or the interaction between stimulus*group. Along this line, ratings within each group were significantly different between the CS+ and the CS- in ACQ1 (HC $t(37) = -8.81$, $p < .001$, SABP: $t(47) = -16.14$, $p < .001$, CBP $t(34) = -13.32$, $p < .001$) and ACQ2 (HC: $t(37) = -12.71$, $p < .001$, SABP: $t(47) = -16.60$, $p < .001$, CBP: $t(34) = -11.11$, $p < .001$) (Fig. 29 and Tab. 31). As expected contingency awareness during EXT did not lead to any significant results, since the US presentation was stopped during this learning phase. All subject samples learned the coupling between the US and CS+ during both acquisitions and that the CS- was never paired to the US. Contingency awareness ratings did not lead to any significant group differences (Fig. 29 and Tab. 32).

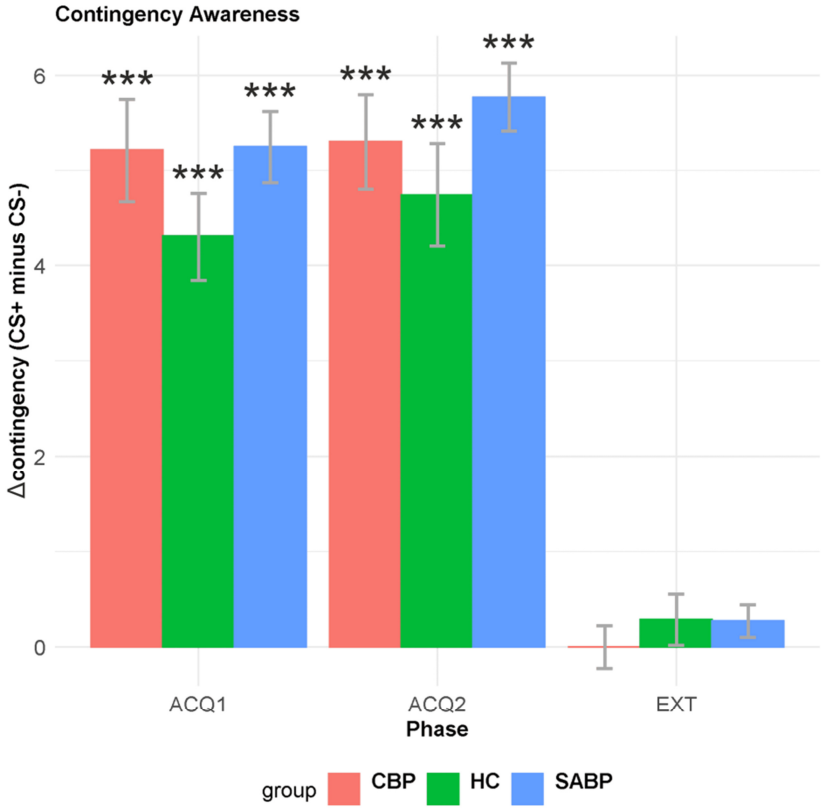

Figure 29: Contingency awareness (CS+ un. minus CS-). Subjective ratings of the contingency awareness (Delta CS ± SEM) in HC (green), SABP (blue) and CBP patients (red) depicted on the y-axis, across different experimental phases (x-axis). Asterisks show significant differences in CS+ versus the CS- ratings within one group (post-hoc t-test, FDR corrected) with significance thresholds *p < .05, **p < .01, ***p < .001. All groups

learned the coupling between CS+ and US during both acquisitions. Group comparisons did not result in significant differences between the subject samples (Tab. 32).

Paired T-Test (within group)

Group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	2.49 *	-9.67 ***	-9.12 ***	-1.07	37
	p	0.05	5.14^{-12}	8.85^{-10}	0.44	
SABP	**t**	2.11	-13.96 ***	-16.14 ***	-1.61	47
	p	0.06	7.30^{-18}	2.55^{-20}	0.34	
CBP	**t**	1.87 *	-10.32 ***	-11.11 ***	0.00	34
	p	0.04	5.14^{-12}	1.12^{-12}	1.00	

Table 31: Statistical test of the contingency awareness ratings. A paired t-test FDR corrected was used to calculate within group differences for the evaluation of the CS+ versus the CS- (* p < .05, **p < .01, ***p < .001) in each group, respectively. During both acquisitions ratings for the CS+ were significantly different from the ratings for the CS-, in all three subject groups. All groups learned the coupling between CS+ and US during ACQ1 and ACQ2, but not during HAB and EXT. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	-0.65	-1.48	-1.52	0.02
	p	0.56	0.36	0.40	0.98
	df	74.36	75.36	64.49	62.17
HC/ CBP	**t**	-1.20	-1.19	-0.69	0.81
	p	0.56	0.36	0.49	0.63
	df	62.15	64.57	66.99	66.16
SABP/ CBP	**t**	0.58	-0.06	-0.76	-0.97
	p	0.56	0.95	0.49	0.63
	df	78.98	61.00	62.32	64.65

Table 32: Statistical comparison of contingency awareness group means. An unpaired t-test (FDR corrected) was used to compare Delta CS arousal levels between subject samples. There were no significant group differences in contingency awareness. t = t-statistics, p = p-value, df = degrees of freedom.

6.2.2 Arousal Ratings

Arousal ratings for the CS+ versus the CS- showed a significant effect of stimulus over all experimental phases (HAB: $F(2,236) = 5.28$, $p = .002$, ACQ1: $F(2,236) = 133.79$, $p < .001$, ACQ2: $F(2,236) = 210.74$, $p < .001$, EXT: $F(2,236) = 52.32$, $p < .001$). There was no significant interaction between stimulus*group. Along this line, arousal levels within each subject group were significantly different for the CS+ versus the CS- across all groups during ACQ1, ACQ2 and EXT (ACQ1: HC $t(37) = -5.12$, $p < .001$, SABP $t(47) = -3.64$ $p < .001$, CBP $t(34) = -6.72$, $p < .001$, ACQ2: HC $t(37) = -7.15$, $p < .001$, SABP: $t(47) = -10.71$, $p < .001$, CBP $t(34) = -8.19$, $p < .001$) (Fig. 30 and Tab. 33). In addition, HC also showed significant different arousal levels for CS+ and CS- after the HAB (Tab. 33). Both pain patients groups showed higher arousal levels in contrast to HC (n.s., Fig. 30 and Tab. 33).

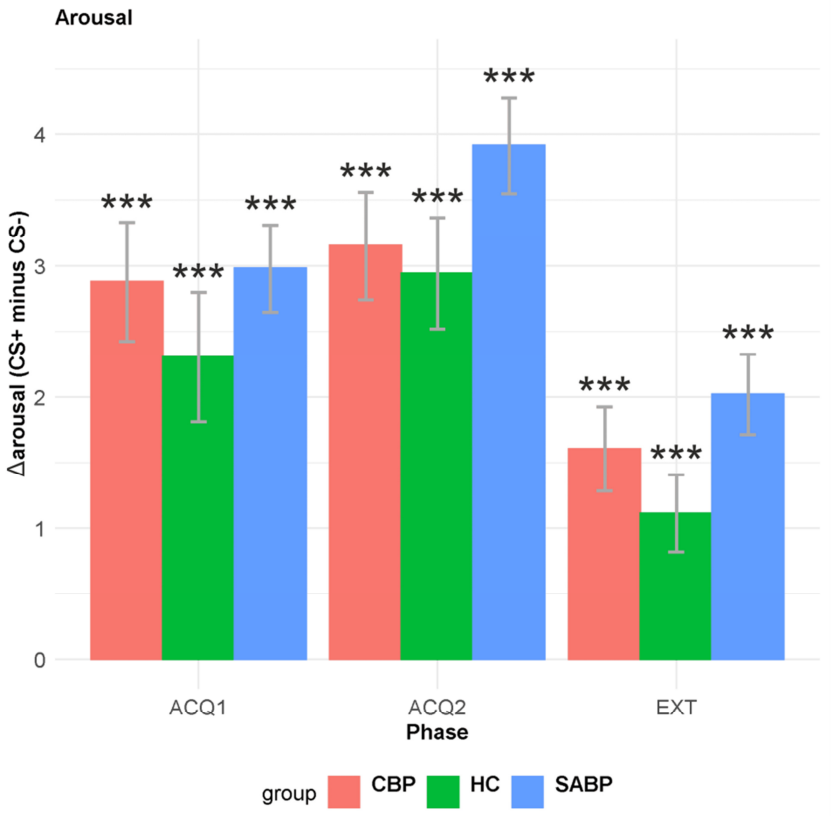

Figure 30: Arousal levels (CS+ minus CS-). Subjective arousal ratings depicted on the y-axis (Delta CS ± SEM) in HC (green), SABP (blue) and CBP patients (red), across the experimental phases (x-axis). Asterisks show significant differences in CS+ versus the CS- ratings within one group (post-hoc t-test, FDR corrected) with significance thresholds *p < .05, **p < .01, ***p < .001. All subject samples rated the CS+ and the CS- significant different throughout the depicted experimental phases. CBP and SABP patients showed higher arousal levels than HC (n.s.). There were no significant group differences in arousal ratings (Tab. 34).

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	3.53 **	-5.12 ***	-7.15 ***	-4.00 ***	37
	p	3.45^{-3}	9.76^{-6}	2.47^{-8}	3.09^{-4}	
SABP	**t**	2.15	-9.00 ***	-10.71 ***	-6.61 ***	47
	p	0.08	2.54^{-11}	9.99^{-14}	9.64^{-8}	
CBP	**t**	-0.21	-6.72 ***	-8.19 ***	0.00 ***	34
	p	0.83	1.50^{-7}	2.22^{-9}	5.58^{-6}	

Table 33: Statistical test of within group comparisons of arousal ratings. A paired t-test FDR corrected was used to calculate within group differences for the evaluation of the CS+ versus the CS- (* p < .05, **p < .01, ***p < .001) in each group, respectively. All subject samples showed significantly different arousal levels for the CS+ and the CS-, across all phases except the HAB. HC were the only group who showed significantly different arousal ratings for both stimuli also after the HAB. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

In addition, there was a significant effect of group during HAB between the HC sample and the CBP patients ($F(2,236) = 6.02$, $p = .002$), as well as between HC and patients with SABP ($F(2,236) = 6.02$, $p = .043$). Post- hoc t-test group comparisons only led to significant differences in Delta CS arousal levels between HC and patients with CBP during HAB (Tab. 34).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	-1.02	-1.08	-1.76	-2.02
	p	0.31	0.64	0.24	0.13
	df	81.08	66.67	76.51	81.20
HC/ CBP	**t**	-2.80 *	-0.80	-0.35	-1.02
	p	0.02	0.64	0.72	0.35
	df	68.00	68.00	66.88	65.76
SABP/ CBP	**t**	1.78	-0.18	-1.39	-0.94
	p	0.12	0.86	0.25	0.35
	df	77.33	62.95	72.01	74.75

Table 34: Statistical comparison of arousal group means. An unpaired t-test (FDR corrected) was used to compare Delta CS arousal levels (*p < .05) between subject samples. There was only a significant group difference in Delta CS arousal levels in the HAB between HC and patients with CBP. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

6.2.3 Valence Ratings

The analyses of the perceived valence ratings during aversive conditioning showed a significant effect of stimulus across all phases and groups (HAB: $F(2,236) = 5.28$; $p < .001$ ACQ1: $F(2,236) = 236.29$, $p < .001$, ACQ2: $F(2, 236) = 314.59$, $p < .001$, EXT: $F(2,236) = 1.32$; $p < .25$), where the US was actually presented. There was only a significant effect of group during HAB between HC and patients with SABP ($F(2,236) = 6.02$, $p = .019$). The interaction between group*stimulus was not significant. Within group comparisons for the aversive CS+ versus CS- ratings resulted in similar results as the ANOVA with significant different valence ratings for both stimuli (CS+ and CS-) in all groups across all phases (ACQ1: HC: $t(37) = -8.11$, $p < .001$, SABP: $t(47) = 8.89$, $p < .001$, CBP: $t(34) = 7.56$, $p < .001$, ACQ2: HC: $t(37) = 7.37$, $p < .001$, SABP: $t(47) = 10.75$, $p < .001$, CBP: $t(34) = 8.85$, $p < .001$) (Fig. 31 and Tab. 35).

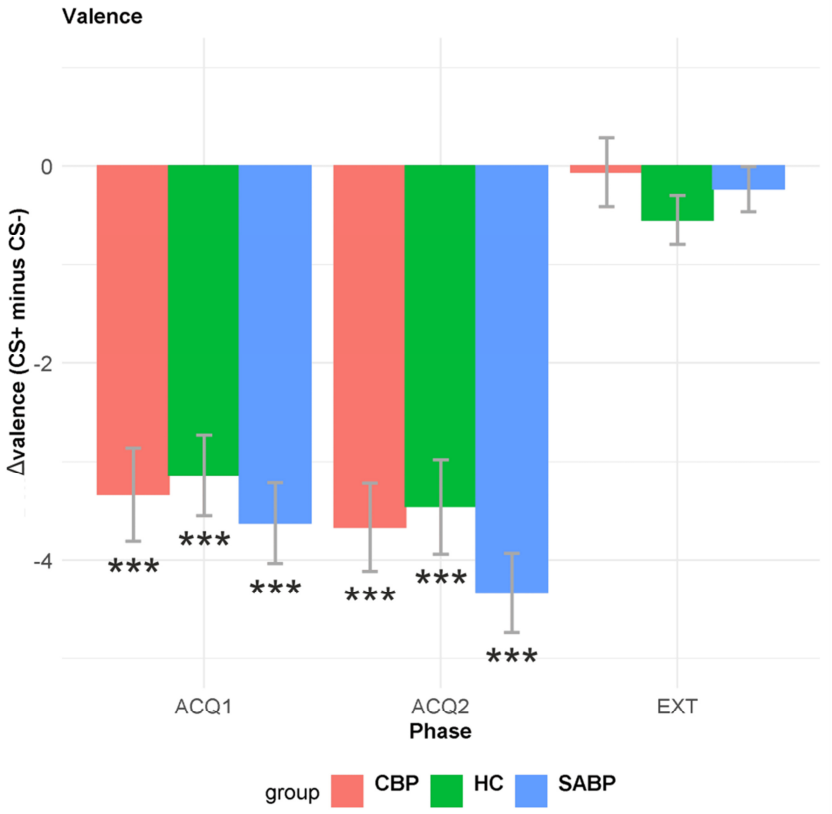

Figure 31: Valence ratings (CS+ minus CS-). Subjective ratings of valence depicted on the y-axis (Delta CS ± SEM) in HC (green), SABP (blue) and CBP patients (red), across the experimental phases (x-axis). Asterisks show significant differences in CS+ versus the CS- ratings within one group (post-hoc t-test, FDR corrected) with significance thresholds *p < .05, **p < .01, ***p < .001. All three subject samples showed significant different valence levels for the evaluation of the aversive CS+ and the CS-, throughout all phases in which the US was presented, but not in the EXT. Patients with SABP and CBP showed lower valence levels than HC (n.s.) in all phases where the US was presented. HC showed lowest valence ratings after the EXT. There were no significant group differences (Tab. 36).

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	-2.91 *	8.11 ***	7.37 ***	2.16	37
	p	0.02	1.48^{-9}	1.26^{-8}	0.11	
SABP	**t**	-1.86	8.89 ***	10.75 ***	1.01	47
	p	0.08	3.74^{-11}	8.88^{-14}	0.47	
CBP	**t**	-1.80	7.56 ***	8.85 ***	0.34	34
	p	0.08	8.72^{-9}	3.59^{-10}	0.73	

Table 35: Statistical test of within group comparisons of valence ratings. A paired t-test FDR corrected was used to calculate within group differences for the evaluation of the CS+ versus the CS- (* p < .05, **p < .01, ***p < .001) in each group, respectively. Valence ratings for the aversive CS+ were significantly different from the CS- in all phases, except the HAB, in all groups. HC showed also in the HAB a significant different valence level for both stimuli. t = t-statistics, p = p-value, df = degrees of freedom, asterisks = significance level.

Valence ratings for the Delta CS were not significantly different between subject samples in neither ANOVA, nor in the post-hoc t-test (Tab. 36).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	1.48	0.81	1.44	-0.90
	p	0.22	0.78	0.41	0.56
	df	62.61	81.94	15.41	78.23
HC/ CBP	**t**	1.74	0.28	0.34	-1.10
	p	0.22	0.78	0.73	0.56
	df	56.54	64.65	67.00	58.30
SABP/ CBP	**t**	-0.30	0.47	1.11	0.41
	p	0.77	0.78	0.41	0.69
	df	78.29	70.57	72.71	57.93

Table 36: Statistical comparison of valence group means. An unpaired t-test (FDR corrected) was used to compare Delta CS arousal levels between subject samples. There was no significant difference between subject groups in their respective Delta CS valence levels. t = t-statistics, p = p-value, df = degrees of freedom.

6.2.4 Skin conductance responses

A significant effect of group was found in SCRs between SABP patients and HC, across all phases except for the HAB (HAB: F(2,178) = 1.33, p = .36, ACQ1: F(2,178) = 5.85, p = .004, ACQ2: F(2,178) = 5.77, p = .005, EXT: F(2,178) = 7.86, p = .003) and between CBP patients and HC (HAB: F(2,178) = 1.33, p = .462, ACQ1: F(2,178) = 5.85, p = .029, ACQ2: F(2,178) = 5.77, p = .021, EXT: F(2,164) = 10.54, p = 001), but not between patient samples (Fig. 32). There was neither a significant effect of stimulus, nor for the interaction of stimulus*group. Within group comparisons for CS+ un. versus CS- SCRs were not significantly different (Tab. 37). SCRs for the aversive CS+ and the CS- showed a similar course within groups throughout the experiment. SCRs descended from HAB to EXT for both stimuli, with HC showing in general highest SCRs for both stimuli (n.s.). The CS+ trials produced higher SCRs than the CS- trials in the SABP sample. HC and CBP patients showed this trend only after the HAB. SCRs for the aversive CS+ and CS- trials during EXT were on a comparable level in HC. CBP patients showed in the HAB lower SCRs for the CS+ than for the CS-, which was switched in the rest of the experiment. SCR levels within in each group, for CS+ and CS- did not yield any significant results between subject samples (Fig. 32 and Tab. 37).

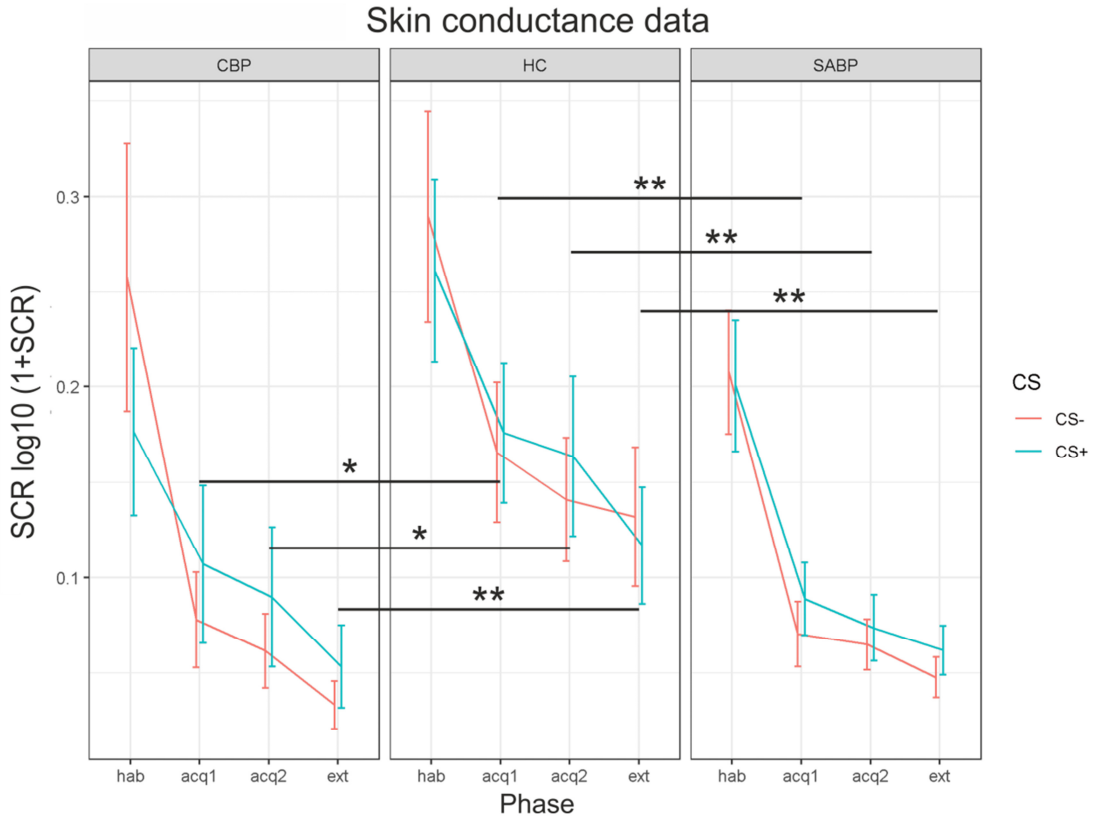

Figure 32: Skin conductance responses (CS+ versus CS-) during aversive conditioning. Log transformed (log10) SCR data (µS) for CS+ responses (blue) and CS- responses (red), depicted on the y-axis, across all four experimental phases (x-axis), for each subject sample separately (subplots). Error bars Delta SCR data ± SEM. Significant effects of group (ANOVA) were marked with straight lines highlighting between which groups and in

which phase of the experiment. Asterisks depict significance threshold (*p < .05, **p < .01). SCRs in all subject groups and in each experimental phase showed a comparable progression of SCRs. SCR levels were significantly different in the ANOVA data, but not in the unpaired t-tests. Both, SABP and CBP patients, showed significant different SCR levels than HC in all phases, except for the HAB.

Paired T-Test (within group)

group		*HAB*	*ACQ1*	*ACQ2*	*EXT*	*df*
HC	**t**	1.33	0.55	-1.73	0.64	27
	p	0.29	0.59	0.25	0.53	
SABP	**t**	0.66	-2.33	-1.06	-2.44	36
	p	0.51	0.08	0.30	0.58	
CBP	**t**	1.36	-1.49	-1.43	-2.00	25
	p	0.29	0.22	0.25	0.09	

Table 37: Statistical test of within group comparison of SCRs for the CS+ and the CS-. There were no significant differences for SCRs for the aversive CS+ and CS- trials, in the paired t-test, across all subject samples and phases of the experiment. t = t-statistics, p = p-value, df = degrees of freedom.

Delta CS SCRs in both ANOVA and unpaired t-tests did not show any significant differences in SCRs between subject samples. Overall Delta CS results for both patient samples showed higher levels of SCRs throughout the experiment in contrast to HC (n.s., Fig. 33). SCRs in both patient samples progressed differently throughout the experiment. In contrast to that, HC showed a descending SCR curve from HAB to ACQ2 and an increase in SCRs during EXT. CBP patients showed the highest SCRs during HAB, descending to ACQ1 and ascending from there on. SABP patients showed the lowest SCRs during HAB and EXT and highest SCRs during both acquisitions. Patients with CBP showed overall the highest SCRs in all phases except for the ACQ1, in contrast to both, the SABP patients and the HC sample (n.s., Fig. 33 and Tab. 38).

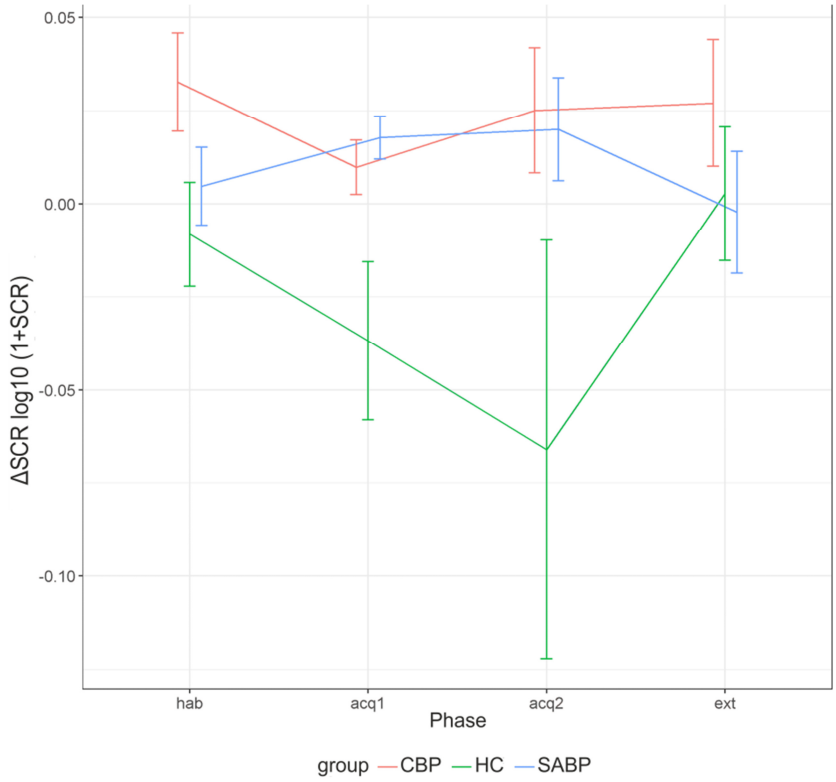

Figure 33: Skin conductance responses Delta CS during aversive conditioning. Log transformed (log10) SCRs (y-axis) for HC (green), SABP (blue) and CBP patients (red), respectively, across all experimental phases (x-axis). Error bars Delta CS ± SEM. CBP patients revealed higher SCRs throughout the experiment than HC and patients with SABP, except during ACQ1 (n.s.). There were no significant group differences in Delta CS SCRs between subject samples (Tab. 38).

Unpaired T-Test FDR corrected

Group comp.		*HAB*	*ACQ1*	*ACQ2*	*EXT*
HC/ SABP	**t**	-0.74	-2.48	-1.48	0.21
	p	0.46	0.06	0.22	0.84
	df	53.42	31.01	30.29	58.68
HC/ CBP	**t**	-2.13	-2.07	-1.55	-0.98
	p	0.11	0.07	0.22	0.49
	df	51.98	33.30	31.76	49.99
SABP/ CBP	**t**	1.66	-0.86	0.23	1.24
	p	0.15	0.39	0.82	0.49
	df	52.24	51.80	53.39	57.14

Table 38: Statistical group comparison of SCRs. An unpaired t-test (FDR corrected) was calculated between all subject samples. There were no significant group differences. t = t-statistics, p = p-value, df = degrees of freedom.

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