Medizinische Fakultät

der

Universität Duisburg-Essen

Aus dem Institut für Medizinische Psychologie und
Verhaltensimmunbiologie

Pain modality shapes neural mechanisms underlying learning and extinction of pain-related fear

Inaugural-Dissertation

zur

Erlangung des Doktorgrades der Naturwissenschaften in der Medizin

durch die Medizinische Fakultät

der Universität Duisburg-Essen

Vorgelegt von

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2018

DOI: 10.17185/duepublico/70716
URN: urn:nbn:de:hbz:464-20191120-095713-7

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Tag der mündlichen Prüfung: 12.09.19

This work was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) within the research unit FOR 1581 "Extinction Learning. Neural Mechanisms, Behavioral Manifestations, and Clinical Implications" (FOR 1581; EL 236/9-2) and the Collaborative Research Center "Extinction Learning" (SFB 1280, Project A10).

Parts of this work have been published as follows:

Publications

Koenen L.R., Icenhour A., Forkmann K., Pasler A., Theysohn N., Forsting M., Bingel U., Elsenbruch S. (2017). Greater fear of visceral pain contributes to differences between visceral and somatic pain in healthy women. PAIN. Aug;158(8):1599-1608. doi: 10.1097/j.pain.0000000000000924.

Koenen, L.R., Elsenbruch, S. (2018a). [Pain is modality-specific: Differences in the perception and processing of interoceptive visceral compared to exteroceptive cutaneous heat pain stimuli]. Schmerz 32, 139-143. doi: 10.1007/s00482-018-0281-y.

Koenen L.R., Icenhour A., Forkmann K., Theysohn N., Forsting M., Bingel U., Elsenbruch S. (2018b). From anticipation to the experience of pain: Neural and behavioral responses to pain-predictive cues are shaped by pain modality. Psychosom Med. [Epub ahead of print]. doi: 10.1097/PSY.0000000000000612.

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1. Introduction

Pain is a multidimensional and highly subjective experience as a reaction to an internal or external noxious input. Pain signals an actual or potential threat to physical integrity and hence represents a salient stimulus that causes distress to the body's homeostasis (Melzack, 2001). Therefore, adaptively learning to identify and to memorize environmental cues that predict painful episodes, serves as an evolutionary-driven strategy to avoid potential injury, restore homeostatic function and sustain health.

In the context of chronic pain, however, maladaptive learning processes are thought to induce negative pain-related emotions, especially pain-related fear, in the anticipation of pain. As conceptualized in the fear-avoidance model of chronic pain (Vlaeyen et al., 2016), the acquisition of these anticipatory negative emotions can lead to maladaptive pain-related behavior, such as excessive avoidance behavior. These processes are assumed to ultimately perpetuate a vicious circle in chronic pain syndromes, by amplifying pain perception, enhancing negative emotions and cognitive distortions, and promoting maladaptive coping strategies (Gatchel et al., 2007). Therefore, conditioned pain-related fear has been acknowledged as a highly relevant factor contributing to the acquisition and maintenance of chronic pain and has gained increasing interest in the past few years as a potentially promising target in cognitive-behavioral pain treatment (Vlaeyen, 2015).

Chronic pain syndromes are characterized by various complex and multifaceted symptoms, which are specific to the given condition. While in chronic low back pain, pain arises from exteroceptive body regions, such as the muscles, skin or joints, other chronic pain syndromes, such as the irritable bowel syndrome or functional dyspepsia, are characterized mainly by interoceptive pain emerging from the viscera. Recently, experimental studies have shed light on different psychophysiological mechanisms underlying distinct pain modalities (Dubin et al., 2010), suggesting specific perception and processing of exteroceptive, somatic compared to interoceptive, visceral pain. However, the neural underpinnings and the potential relevance of modality-specific pain aspects for the acquisition of distinct chronic pain syndromes have not been thoroughly explored yet. Specifically, the role of pain modality in learning and extinction of pain-

related fear has never been addressed. Therefore, the overarching aim of this dissertation was to examine if and how modality-specific pain characteristics, based on their underlying distinct psychophysiological mechanisms, can mediate pain-related fear learning and memory processes in healthy adults.

To this end, two consecutive experimental studies were accomplished. In the first study, joint and differential neural responses were examined to visceral compared to somatic pain stimuli (Koenen et al., 2017) and to pain-predictive cues (Koenen et al., 2018b). Built on the methods and results from study 1, modality-specific effects on learning, extinction and reactivation of pain-related fear were tested in the second study, using a differential fear conditioning paradigm with visceral and somatic pain as competing unconditioned stimuli.

1.1. Psychophysiological mechanisms of visceral and somatic pain

1.1.1. The multidimensional experience of pain

The experience of pain is more than just a simple response to nociceptive input (Mayer et al., 2015). Rather, pain is mediated by several interacting factors, encompassing sensory-discriminative, emotional-affective and cognitive-behavioral aspects, as described within the biopsychosocial model of pain (Gatchel et al., 2007; see Fig.1). These factors can modulate associated expectations regarding the intensity, duration or controllability of pain, adaptively shape pain perception and behavior and thus largely contribute to the individual pain history.

Moreover, the experience of pain is not static, but can undergo changes. Altered expectations or learning, as well as endogenous pain modulatory mechanisms (habituation/sensitization) can modulate pain perception, leading to an increasing or decreasing perception of pain over time. Exceedingly increased sensitivity or responsivity to pain, i.e., hyperalgesia, which can be the result of severe acute and/or ongoing injuries, is referred to as one of the core symptoms of chronic pain (Gebhart, 2000). Furthermore, the emotional (re-)evaluation of painful events as being increasingly more unpleasant, disturbing, or fear-inducing, does also seem to play a putative role in the experience of painful episodes and contributes to the individual level of suffering or agony resulting from pain (Elsenbruch, 2011).

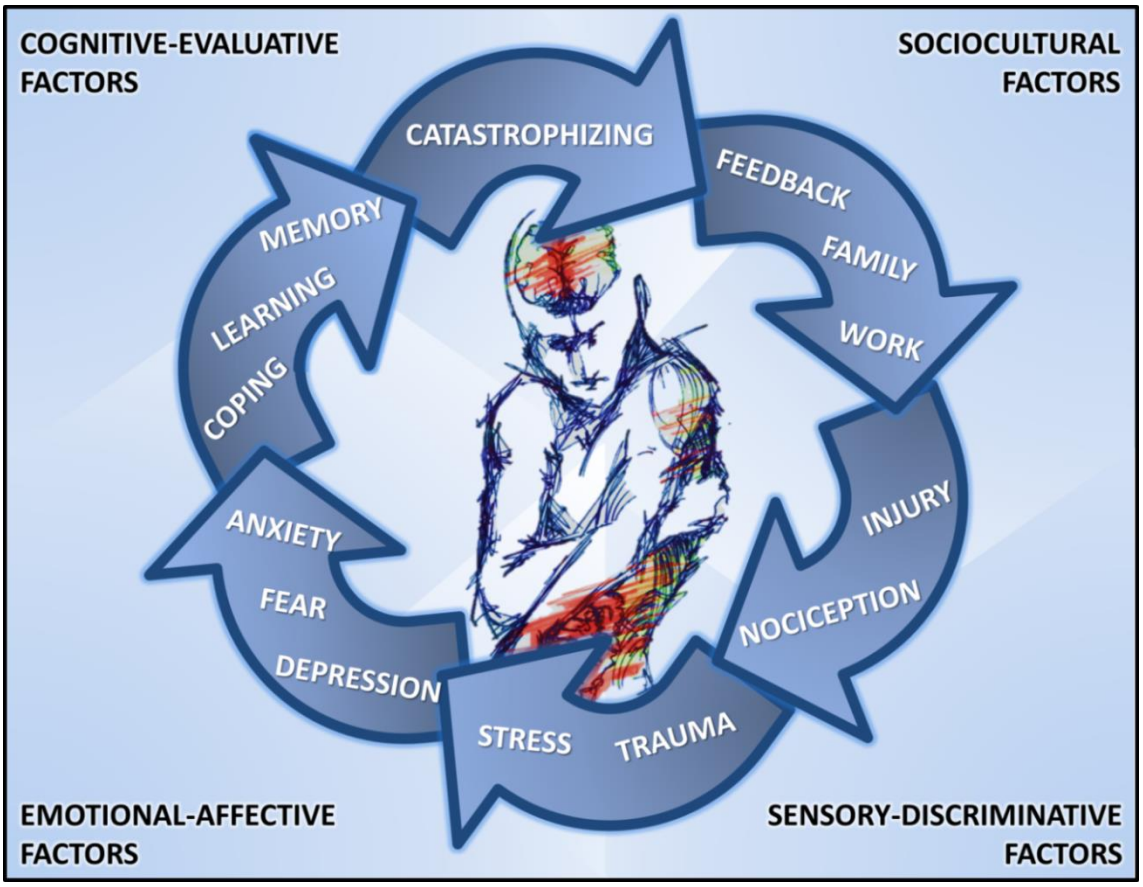

Figure 1: The biopsychosocial model of pain. Pain perception and processing are affected by the modulatory impact of cognitive-evaluative, sociocultural, emotional-affective and sensory-discriminative factors. While differences in peripheral nociception between visceral and somatic pain have been described, modality-specific aspects in modality-specific processing of pain and especially, the putative role of psychological aspects, such as pain-related fear learning and memory, have not been thoroughly explored yet. Adapted from Gatchel (2007).

Unsurprisingly, patients with chronic pain conditions often also suffer from comorbid emotional-affective disturbances, such as anxiety or depression (Gatchel et al., 2007), which have to be considered during treatment. Therefore, emotional-affective aspects of pain perception have gained increasing attention in the past years as an important characteristic of the pain experience.

Interestingly, the perception of pain seems to be also determined by the body area, from which the pain originates. For instance, the perceived characteristics of dental, stomach or muscle pain are described as fundamentally different, albeit they all involve the feeling of pain or aversiveness. Especially between interoceptive, visceral and

exteroceptive, somatic pain, several differences in perception of painful stimulation have been reported. As reported by acute and chronic pain patients alike, visceral pain is perceived as diffuse and poorly localizable (Cervero, 2009). Moreover, the perception of visceral pain often spreads across visceral as well as associated somatic areas, a phenomenon called referred pain (Gebhart et al., 2016), while somatic pain can be more easily associated with a specific body region. While these findings suggest differences in the perception of sensory-discriminative pain aspects across pain modalities, visceral pain also seems to be unique with regard to emotional-affective pain characteristics: In one of the few studies directly comparing modality-specific aspects of pain perception, visceral pain stimuli were revealed to be equally unpleasant compared to somatic pain at significantly lower pain intensities (Dunckley et al., 2005). The feeling of higher unpleasantness with regard to visceral pain may be attributable to its interoceptive nature. Given the restricted visibility and accessibility of visceral stimuli, the actual impact and potentially fatal consequences of visceral pain are possibly harder to evaluate, which may thereby contribute to the higher discomfort and/or anxiety.

Different chronic pain syndromes, such as fibromyalgia, chronic low back pain, chronic pelvic pain or irritable bowel syndrome (IBS), are characterized by pain originating from different body regions. Therefore, differences in emotional-affective and sensory-discriminative aspects of pain perception across pain modalities are likely to characterize pain-related symptoms specific for the given condition. However, if and how modality-specific aspects contribute to the pathology underlying different chronic pain syndromes is still unclear. Given the unique characteristics of visceral compared to somatic pain, it seems also likely that pain modality shapes the experience of acute pain, rendering the role of pain modality a relevant, but as of yet neglected factor in acute and chronic pain research.

1.1.2. Modality-specific mechanisms: From the periphery to the brain

Physiologically, body regions are often classified as either one of (at least) two distinct pain modalities: Visceral or somatic pain. While visceral pain is referred to interoceptive, nociceptive input emerging from internal organs, such as the gut, colon, bladder or heart, exteroceptive, somatic pain is thought to originate in skin, joints or muscles. This classification might appear oversimplified, given that some of the deeper

muscular tissues termed as somatic might be technically interoceptive as well. Still, there is accumulating evidence that nociceptive signals emitted from visceral areas are unique, and differ not only in peripheral, but also in central processing (Cervero, 2009), from all other body tissues labeled as somatic (Fig.2).

In general contrast to somatic pain, visceral nociceptive signal processing is characterized by complex interactions along the bidirectional brain-gut axis (Elsenbruch, 2011; see Fig.2E), including processes of the local microenvironment (Cervero, 2009), the gut mucosa, as well as activation of the central nervous system (CNS) and enteric nervous system (ENS) (Mayer et al., 2011). For instance, neuro-immune communication along the brain-gut axis together with stress mechanisms involving hypothalamic-pituitary-adrenal axis (HPA-axis) activation is suggested to modulate visceral hyperalgesia (Elsenbruch, 2011). The ENS is a specialized autonomous nerve system which regulates elementary functions of the GI-tract, such as motility, blood flow and secretion (Gebhart et al., 2016). Although possible interactions between the ENS and other parts of the peripheral nervous system have been suggested for instance via the vagus nerve (for details see Gebhart et al., 2016), the exact reciprocal mechanisms are unknown and the potential contribution to conscious pain perception is still unclear (Gebhart et al., 2016).

In both pain systems, noxious information is initially received by spinal afferents in the periphery (Fig.2A, B) and transferred either by uni- or polymodal A-fibers and C-fibers into respective areas of the spinal cord (Dubin et al., 2010), which convey either specific or combined noxious input (mechanical, thermal and/or chemical). In the somatic system, fiber types can be linked more directly to aspects of pain perception. While some fibers promote slow, dull pain (C-fibers), other fibers rather convey sharp, quick pain sensations (Aδ-Fibers) (Almeida et al., 2004). Moreover, in some exteroceptive body tissues, i.e., the human skin, ion channel sensors have been identified that were able to be associated with a specific input type, i.e., a chemical agent (for an overview see Dubin et al., 2010). For the visceral modality, the picture seems way less clear. It has been suggested (Gebhart, 2000) that there is a broad and complex range of free nerve endings, i.e., nociceptors, with a decreasing shift of distribution and complexity of spinal afferents from upper (esophagus, gut area) to lower (colon, rectum) parts of the gastrointestinal tract (GI-tract). Specifically, at least

five structurally distinct free nerve endings of thinly myelinated C- and Aδ- fibers have been identified in the human gut and data of animal studies suggest at least thirteen different types of free nerve endings in the colorectum/pelvic area (For more details on free nerve endings in the viscera, see Gebhart, 2000; Gebhart et al., 2016). Recent evidence has suggested that clinical symptoms of functional gastrointestinal disorders, such as visceral distensions caused by bloating, may be associated with Aδ- and C-fiber activation in the anal mucosa (Jones, 2012). However, this data is largely based on animal studies and the exact function and interaction of the various free nerve endings in the viscera remains insufficiently understood.

For the somatic as well as for the visceral pain system, nociceptive information received within the periphery is transmitted via C-fibers to superficial laminae I and II, and via A-fibers to superficial laminae I and V of the dorsal horn (for an overview see Benarroch, 2016), where the peripheral first order neurons synapse with second order afferent neurons (Cervero et al., 1999; Dubin et al., 2010; Gebhart et al., 2016; see Fig.2C). Notably, the overall quantity of spinal nerve afferents conveying nociceptive information into the dorsal horn is lower and less distributed within the visceral compared to the somatic pain system, with only 5-15% of spinal input arising from the viscera (Gebhart et al., 2016). This is viewed as one of the potential reasons why visceral pain is perceived as more diffuse and more difficult to localize compared to somatic pain (Cervero, 2009). Interestingly, second order afferent neurons, especially in lamina I, receive convergent input from dorsal root ganglia innervating visceral and somatic body areas. This convergent input leads to the sensation of "referred pain" (Gebhart, 2000; Gebhart et al., 2016) and can be linked to several clinical symptoms, such as referred hyperalgesia (Cervero, 2009; Gebhart et al., 2016). Within the dorsal horn, different types of excitatory and inhibitory projection neurons and interneurons are engaged, which interact in amplifying and depressing innocuous and noxious input from first to second order afferent neurons (Benarroch, 2016). Some projection neurons in the dorsal horn, so-called "wide dynamic range neurons" (WDR-neurons) are assumed to display an increasing frequency-based excitability due to repeated nociceptive input, termed "wind-up" (Benarroch, 2016). "Wind-up" is reportedly associated with short- up to medium-term hyperalgesia and has been reported to be different for dorsal horn neurons receiving somatic compared to neurons receiving

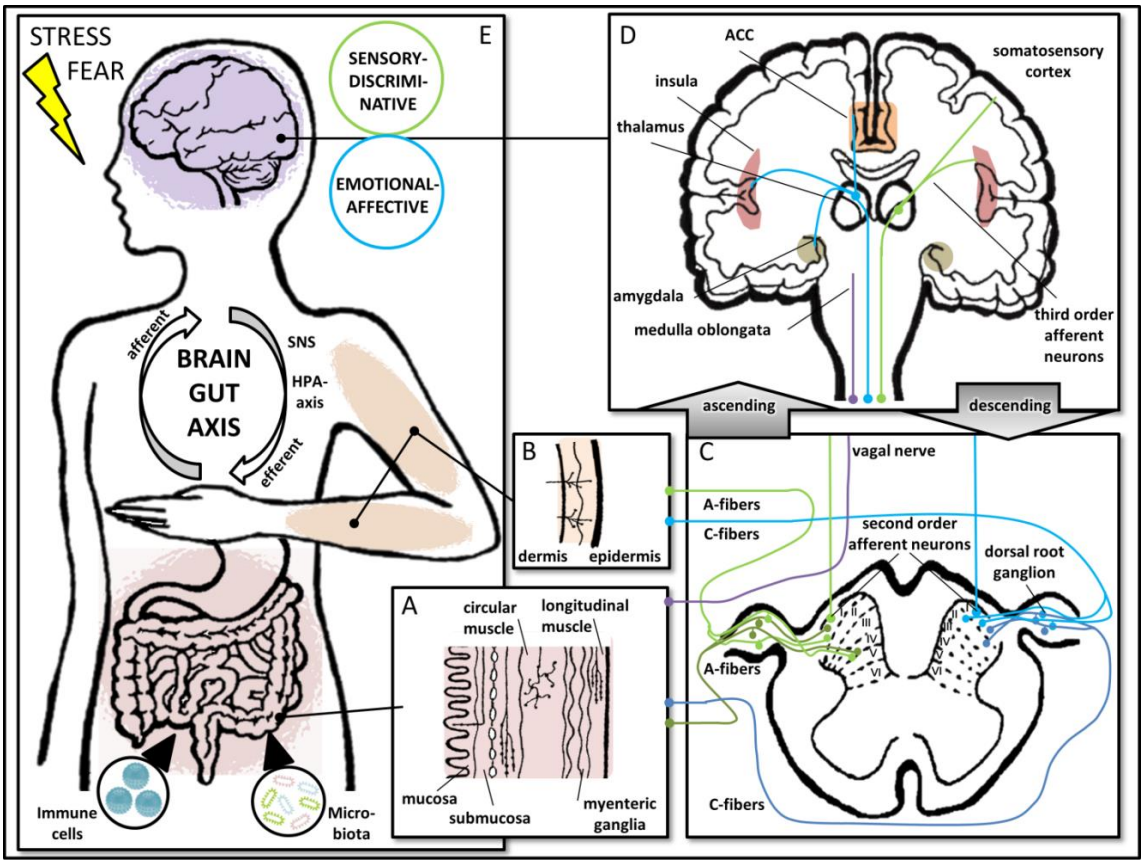

Figure 2: Peripheral, spinal and neural levels of visceral and somatic pain processing. Differences in ascending pain signal transmission are displayed for peripheral afferent input, i.e. distinct types of free nerve endings for the visceral (here colon, A) compared to the somatic modality (here skin, B). Differential processes have also been suggested in the dorsal horn of the (C) spinal cord and (D) in brain areas associated with pain. Moreover, pain perception and processing in both modalities is strongly mediated by excitatory or inhibitory descending nociceptive control mechanisms (not shown here in detail) on different levels of pain processing. Furthermore, for visceral pain, pain perception and processing is modulated by afferent and efferent communication along the (E) brain-gut-axis, as well as the specific microbiotic environment, neuro-immune interactions, and potentially signal transmission via the vagal nerve. (Adapted from depictions in Almeida 2000, Dubian & Papapoutian 2010; Gebhardt & Bielefeld 2011; Elsenbruch, 2011). Abbreviation: ACC, anterior cingulate cortex; HPA-axis, hypothalamic-pituitary-adrenal-axis; SNS, sympathetic neural system.

viscero-somatic nociceptive input (Cervero et al., 1999). Hence, endogenous pain sensitization processes mediated by spinal mechanisms suggested to be involved in the acquisition of chronic pain (Sandkuhler et al., 2012), may differ across pain modalities.

From the dorsal horn, noxious information received by projection neurons and WDR-neurons is transmitted via ascending spinal pain pathways (for an overview, see Almeida et al., 2004) to different nuclei in the thalamus, as well as directly to other subcortical and cortical structures, where they synapse with third order neurons. Specifically, from the lateral nuclei of the thalamus, third order neurons project into the primary and secondary somatosensory cortex, as well as into the posterior insula as part of the lateral pain system, linked to processing of sensory-discriminative pain aspects. Projections from the medial nuclei reach into the anterior cingulum, the prefrontal cortex and the amygdala and are subsumed in the medial pain system, associated with processing of affective-motivational pain qualities (Kulkarni et al., 2005; Fig.2D). Input in the cortex via ascending pain pathways, and thus the subjective experience of pain, is also modulated by activity of descending pain pathways via excitatory or inhibitory nociceptive control mechanisms, which can facilitate or inhibit the intensity of incoming nociceptive information, respectively. These descending pathways mainly involve the brainstem, but are also mediated in a top-down fashion by other cortical brain regions (Heinricher et al., 2009).

1.1.3. Modality-specific mechanisms: The brain in pain

For decades, researchers have attempted to define a cortical basis for pain. In recent years, multimodal brain imaging studies have provided further insights into pain processing on neural levels, revealing consistent activation in response to nociceptive input in an extensive array of cortical and subcortical areas. These areas include the primary and secondary somatosensory cortex, the insular and cingulate cortex, the thalamus, limbic areas and prefrontal structures (Iannetti et al., 2010), which are not only involved in processing of acute pain, but also show altered activation in chronic pain patients (Apkarian et al., 2011). Based on these findings, proposals have been made to either define a specific “neuromatrix of pain” (Melzack, 2001; Moisset et al., 2007) or, more recently, a “neurosignature of pain” (Wager et al., 2013). However, the majority of the studies included in these reviews report pain processing based on stimulation of somatic, exteroceptive body regions. Moreover, many of the areas included in such an allegedly pain-specific neural system cannot be exclusively associated with pain processing, but are also involved in a number of other processes (Iannetti et al., 2010), such as social decision making (Lockwood et al., 2018), emotion

regulation (Langner et al., 2018), or self-awareness (Craig, 2009). Especially, there has been an ongoing discussion regarding the exact function of the anterior insula and the anterior cingulate cortex (ACC). While these areas have been traditionally included into the neural pain system (Melzack, 2001), other authors argue that they rather display core regions of a brain network related to processing of salience aspects (Mayer et al., 2015; Menon et al., 2010; Wiech et al., 2010), also, but not exclusively, in the context of pain.

Interestingly, there seems to be an overlap between some brain areas associated with pain processing and regions displaying neural activation during events predicting pain. Thalamic, insular and cingulate regions (Palermo et al., 2015) have also been reported to show altered activation during pain processing and pain anticipation in chronic pain patients (Brown et al., 2014; Lloyd et al., 2016; Mayer et al., 2015). Imaging studies have demonstrated that these anticipatory neural responses were able to modulate the pain response (Berman et al., 2008; Palermo et al., 2015; Seifert et al., 2013), suggesting a functional relation between the neural networks involved in pain processing and learned emotional responses in the anticipation of pain with potential relevance for chronic pain processes.

In past years, there have been attempts to address the questions how the brain identifies and distinguishes visceral from somatic pain (Aziz et al., 2000; Dunckley et al., 2005; Mayer et al., 2015; Strigo et al., 2003), and there is first evidence that modality-specific aspects of pain are reflected on neural levels. For visceral pain, studies have demonstrated activation in the ACC and the insula, as well as the primary somatosensory cortex, posterior parietal cortex, prefrontal regions and the thalamus (Mayer et al., 2015), i.e., in similar areas involved in pain processing induced by somatic pain (Melzack, 2001; Moisset et al., 2007). Therefore, differences across modalities may not be displayed in entirely distinct, but also overlapping neural networks, which perhaps differ in the extent of neural activation to visceral compared to somatic pain. Indeed, a few studies directly comparing visceral and somatic pain have provided first evidence of differences in neural activation between pain modalities within somatosensory cortices and cingulate cortices when esophageal distensions were compared to anterior chest wall heat pain induction (Strigo et al., 2003) or between the proximal and distal part of the esophagus in males (Aziz et al., 2000). Moreover, when

stimuli were matched for unpleasantness, higher deactivation of the perigenual anterior cingulate cortex (pgACC) was observed for electrical rectal compared to abdominal wall stimulation (Dunckley et al., 2005). Notably, the only study directly comparing visceral and somatic nociception between patients with IBS and healthy controls was also able to demonstrate altered neural responses in the anterior insula and the mid- and anterior cingulate cortex (Verne et al., 2003), suggesting that brain areas demonstrating differential activation across pain modalities also show alterations in chronic pain. These findings strongly suggest differential visceral and somatic pain mechanisms, especially in brain areas associated with emotional-affective and salience aspects of pain processing.

1.2. Learning and extinction of pain-related fear

1.2.1. Pain-related fear: Learning processes in classical conditioning

From an evolutionary perspective, learning to associate stimuli and situations with the threat of incoming pain and discriminate them from those that are not threatening, is an adaptive skill required to preserve a subject's safety and ultimately better the chances for survival (Pittig et al., 2018; Vlaeyen, 2015). In recent years, these associative learning mechanisms have been widely acknowledged and extensively studied as a key process in acquisition, extinction and return of fear in anxiety disorders and phobias (Pittig et al., 2018). Based on this research, the translational fear-avoidance model of pain (Vlaeyen et al., 2016) was developed, in order to explain pathological pain-related behavior in patients with chronic pain. According to this influential model, the acquisition of fear of relevant cues that have predicted pain in the past and will potentially predict pain in the future is a highly adaptive process aimed at minimizing the chance of experiencing further painful episodes in order to preserve physical integrity (Vlaeyen et al., 2016). However, when pain-related fear becomes excessive or generalized, it can lead to distorted cognitions and negative emotions also in non-threatening situations, erroneously believed to predict pain. This maladaptive associative learning process is then proposed to promote excessive avoidance behavior and social withdrawal, which can severely limit the patient's life quality (Vlaeyen, 2015).

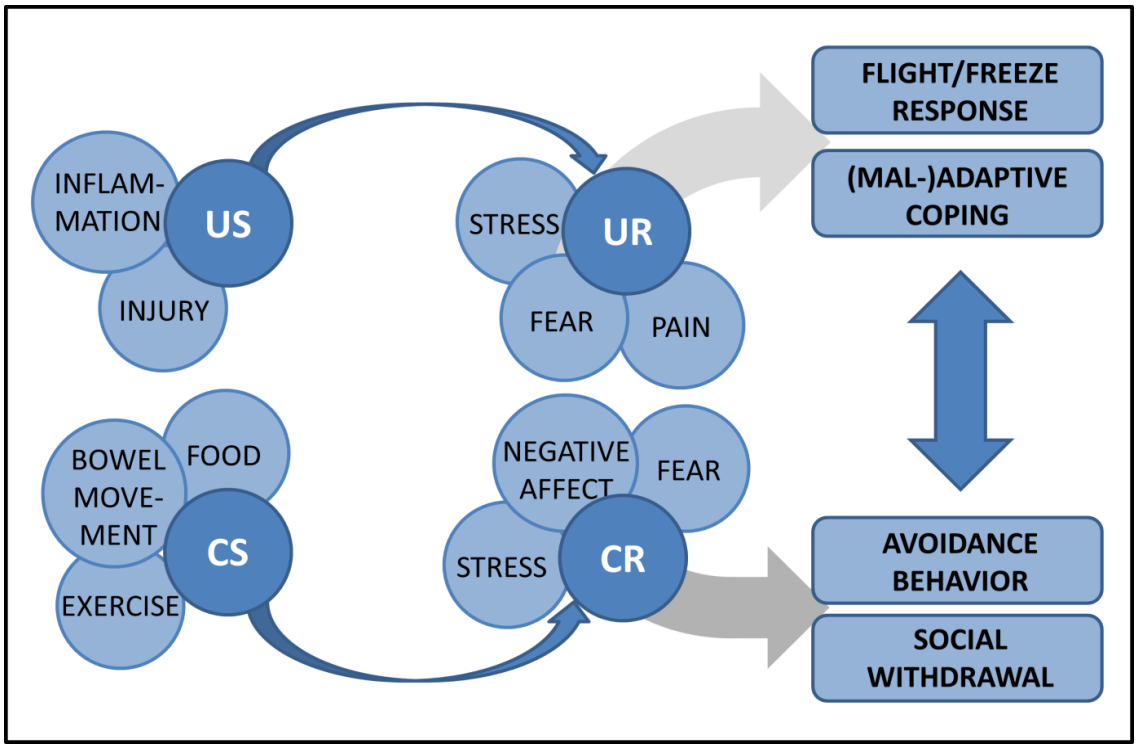

Figure 3: The fear-avoidance model. Unconditioned aversive stimuli or events (US), can induce reflexive behavioral and physiological fear and stress responses, termed unconditioned responses (UR), which can lead to direct physiological fight/flight responses and to long-term maladaptive coping strategies. After repeated pairing with the unconditioned stimulus (US), a former neutral stimulus or event is associated with the US and thus becomes the conditioned stimulus (CS) prompting anticipatory conditioned responses (CR) similar to the original UR via associative learning. Eventually, these associative learning processes promote maladaptive changes in behavior contributing to a loss in quality of life. Adapted from Vlaeyen (2015).

The acquisition of pain-related fear in the fear-avoidance model is based on the well-established learning paradigm of Pavlovian conditioning. Pavlovian conditioning has been originally defined as learning of an association between two formerly unrelated stimuli (see Fig.3): A neutral stimulus (NS) and an unconditioned stimulus (US) with an intrinsic value, which can be either attractive or aversive, prompting reflexive behavioral and/or autonomic physiological reactions, termed the unconditioned reaction (UR) (Pavlov,1927). In the context of fear conditioning, the US (e.g. pain stimuli, loud noise, air puff, etc.) consists of an innate threatening, fear-inducing value, reflected by behavioral fear responses, i.e., freezing or flight, as a UR. After repeated pairing with the US during acquisition learning, the formerly neutral stimulus acquires a predictive value to the US, thereby becoming the conditioned stimulus (CS).

As a result, the CS alone is able to induce fear responses in anticipation of the US, termed conditioned reactions (CR), which are similar to the UR. In most fear conditioning studies, conditioned responses to a pain-predictive CS^+ are often measured in comparison to a non-pain predictive CS (CS^-), which was never paired with the US during acquisition and thus does not acquire an aversive value (Büchel et al., 2000). The learning curve during acquisition, i.e., the speed, as well as the strength of the conditioned responses, is determined by CS and US characteristics (stimulus intensity or type) as well as by other aspects of the conditioning procedure, such as the reinforcement schedule, number of presentations, or CS-US timing (Rescorla et al., 1972).

Several experimental studies have demonstrated (as reviewed by Sehlmeyer et al., 2009) that pain-related fear can be established as a CR in the anticipation of a painful US in humans, measurable on a perceptional level based on subjective ratings and by physiological parameters. Traditionally, the acquisition and extinction of conditioned fear responses is measured by changes in physiological responses (i.e., electrodermal activity (EDA) or heart rate) or autonomic reflexes (e.g. eye blink or startle reflex) based on animal models (Büchel et al., 2000). In humans, also verbal ratings of emotional and cognitive aspects of learning are assessed. In addition to pain-related fear, ratings of CS-induced unpleasantness (usually termed as CS valence) or perceived CS-US contingency awareness are often used as read-out parameters of the conditioned response (Büchel et al., 2000).

In recent years, multimodal imaging studies have additionally provided insights into the neural underpinnings of associative learning of pain-related fear (Büchel et al., 2000; Sehlmeyer et al., 2009). In this regard, several core brain regions were identified to be reliably involved in acquisition of learned fear, such as the insular and cingulate cortex and the amygdala (Büchel et al., 2000). Furthermore the hippocampus, striatum, thalamus and the cerebellum have found to be engaged (Sehlmeyer et al., 2009), however, activation in these regions is seemingly also dependent on the given conditions of the fear conditioning procedure and the stimuli involved (Sehlmeyer et al., 2009). Some studies have already demonstrated that neural activation in these areas during acquisition learning is altered in patients with visceral (Icenhour et al., 2015; Labus et al., 2013) as well as somatic chronic pain conditions (Lloyd et al., 2016),

substantiating the relevance of these neural networks for pathophysiologic processes in chronic pain. While the acquisition of fear responses has inspired various experimental studies in animals and humans alike (albeit open questions remain), the neural basis underlying extinction learning is still insufficiently and incompletely understood.

1.2.2. Neural basis of extinction and return of pain-related fear

During extinction learning, the CR can equivalently be trained to be inhibited by repeatedly presenting the CS without subsequent application of the US. This process has been demonstrated to induce a measurable reduction or omission of learned CR to the CS (Hermans et al., 2006; Pavlov, 1927). Based on the concept of extinction learning, modern cognitive-behavioral approaches of chronic pain therapy repeatedly expose patients to allegedly pain-predictive situations or stimuli with a minimized risk of experiencing actual pain (Vlaeyen et al., 2012). Thereby, an extinction of learned pain-related fear responses is expected and thus a reduction of maladaptive pain-related behavior (Vlaeyen et al., 2012). First evidence supports that this approach is effective in patients with chronic pain conditions characterized by somatic (Linton et al., 2008) as well as by visceral pain (Craske et al., 2011; Ljotsson et al., 2014).

However, extinction learning is a complex and fragile process involving both forgetting of learned aspects as well as new learning of fear-inhibitory memories (Bouton, 2004). Moreover, once acquired fear memories can be re-established even after allegedly successful extinction. This is most likely due to the persistence of an underlying CS-US association that is not erased, but rather suppressed by the new inhibitory memory trace learned during extinction (Bouton, 2004). In experimental studies, fear responses have been demonstrated to return spontaneously (spontaneous recovery), or after a change of context linked to past pain experiences (renewal) as well as in consequence to unpredicted painful events, i.e. unexpected US presentation (reinstatement) (Haaker et al., 2014). In patients with chronic pain disorders, the probability of perceiving unexpected re-occurring painful episodes during curative treatment of chronic pain is expectably high. Therefore, a return of fear becomes highly likely, which increases the probability of a re-installment of maladaptive cognitive processes and disabling behavior (Haaker et al., 2014). Since painful events seem neither predictable nor controllable for the pain patient, helplessness and frustration increase, further decreasing

the chance of successful pain therapy. Therefore, the factors and circumstances involved in the return of fear due to reinstatement after allegedly successful extinction of fear responses are of particular interest in order to increase the effectiveness and long-term validity of chronic pain therapy.

However, especially the neural mechanisms underlying the extinction and the return of fear remain incompletely understood. In human fear conditioning, the hippocampus, the amygdala and ventromedial prefrontal regions (vmPFC), the so-called "extinction network", were found to be engaged during extinction (Fullana et al., 2018; Hermans et al., 2006) as well as during the return of pain-related fear (Icenhour et al., 2015; Lonsdorf et al., 2014). According to the most recent review addressing the neural correlates of extinction learning and extinction recall in differential fear conditioning (Fullana et al., 2018), brain areas engaged in extinction learning are, however, not limited to this network. Rather, a variety of brain regions is proposed to be involved, spanning from dorsal and ventromedial prefrontal areas over to cingulate and insular regions, also including the striatum, the midbrain and dorsal pons (Fullana et al., 2018). Similarly, far more regions might be involved during return of fear after reinstatement, but the exact neural networks remain to be investigated.

Experimental studies have suggested that even a few visceral (Icenhour et al., 2015) as well as somatic pain stimuli (Lonsdorf et al., 2014) suffice to reactivate fear responses on behavioral and neural levels. Therefore, the reactivation of fear responses seems to be a risk factor for chronic pain therapy, independent of the pain modality involved. Henceforth, a better understanding of behavioral and neural mechanisms underlying reinstatement and inter-individual differences in the return of fear seems necessary in order to elucidate the processes underlying the transition from acute to chronic pain and ultimately improve the long-term success of both somatic and visceral chronic pain treatment.

1.2.3. A role of pain modality in pain-related fear learning

Existing knowledge about the cortical basis of pain-related fear learning is mainly based on fear conditioning studies using somatic pain stimuli as US. Of note, only a small share of studies included in current reviews about neural activation during fear acquisition (1/46 in (Sehlmeyer et al., 2009) and extinction (3/31 (Fullana et al., 2018))

have used a visceral pain stimulus as the US. Hence, the neural mechanisms underlying visceral pain-related fear learning remain less well understood.

In recent years, however, the role of visceral pain in fear conditioning has gained increasing interest. Experimental studies have begun to elucidate the specificity of visceral pain in the acquisition and extinction of learned behavioral and neural pain-related fear responses. These studies have demonstrated that pain-related fear learning can be successfully established using classical conditioning with visceral pain stimuli as US (Ceunen et al., 2016; Gramsch et al., 2014; Icenhour et al., 2017; Icenhour et al., 2015; Labrenz et al., 2016) as indicated by accurate contingency awareness, enhanced negative valence ratings (i.e., higher unpleasantness), and higher skin conductance responses to pain-predictive CS^+ compared to non-pain-predictive CS^-. On a neural level, enhanced activation in the putamen (Gramsch et al., 2014), insula (Icenhour et al., 2017), somatosensory regions (Gramsch et al., 2014; Yaguez et al., 2005), and ACC (Yaguez et al., 2005) was reported to visceral pain-predictive CS^+ during acquisition. During extinction, neural responses in the cingulate cortex and sensory-motor regions were revealed (Gramsch et al., 2014) and return of fear was observed engaging the hippocampus, amygdala and vmPFC (Gramsch et al., 2014; Icenhour et al., 2015). Arguably, these studies suggest that some brain areas engaged during the acquisition and extinction of conditioned visceral pain-related fear (Gramsch et al., 2014; Icenhour et al., 2017; Icenhour et al., 2015; Labrenz et al., 2016) are similar to cortical regions reported for somatic pain-related fear learning (Haaker et al., 2014; Lonsdorf et al., 2014; Meulders et al., 2012b). However, differences in pain-related fear learning and memory processes across pain modalities remain unclear.

Based on its unique psychophysiological characteristics especially with regard to emotional-affective pain aspects, visceral pain is suggested to be of higher salience compared to exteroceptive somatic pain. Per definition, salience is a stimulus property that makes one event more distinctive, more conspicuous compared to its surrounding (Legrain et al., 2011). Albeit pain itself constitutes a highly salient event that requires immediate attention, visceral pain stimuli may be considered as more threatening compared to somatic pain, since its potentially fatal consequences are harder to evaluate, increasing the need to avoid visceral pain experiences in advance. This aspect is captured by the preparedness theory (Ohman et al., 2001), which postulates that

stimuli of higher innate salience are learned preferentially in order to quickly and adaptively establish defensive or avoidance behavior and most efficiently manage given resources. In the context of pain-related fear conditioning, pain stimuli of higher innate salience are therefore expected to be associated faster and prompt more negative emotional responses after successful acquisition of the CS-US association when directly compared to less salient stimuli (Ohman et al., 2001). Moreover, extinction of pain-related fear responses is also expected to be less robust and more prone to a return of conditioned fear responses for the more salient stimulus, in order to quickly adapt to external changes in relevant cues or situations.

In sum, these theoretical considerations imply that pain-related fear learning and memory processes are mediated by modality-specific pain characteristics based on the supposedly higher salience of visceral compared to somatic pain, which remain yet thus far experimentally untested in healthy adults.

2. Aims and hypotheses

Taken together, there is emerging evidence for distinct psychophysiological mechanisms underlying visceral and somatic pain on peripheral and potentially neural levels, which presumably shape differential perception between pain modalities. However, only a few studies have directly compared perception and neural processing across pain modalities systematically, especially with respect to differences in sensory-discriminative and emotional-affective pain aspects. Therefore, one aim of this thesis was to elucidate similarities and differences in pain perception and processing between pain modalities.

Furthermore, the neural basis underlying the acquisition and extinction of pain-related fear remains incompletely understood, especially for the visceral modality, and the potential relevance of modality-specific pain aspects in pain-related fear conditioning has not been examined yet. Based on the concept of the preparedness theory, the supposedly higher salience of visceral compared to somatic pain is expected to mediate pain-related fear learning and memory processes. Therefore, the second aim of this thesis was to examine the role of pain modality in pain-related fear learning and extinction in order to elucidate mechanisms relevant for the acquisition and maintenance of distinct chronic pain syndromes shaped by visceral or somatic pain, respectively.

To address these aims, two consecutive functional magnetic resonance imaging studies were conducted in healthy adults: In the first study, joint and differential neural responses to visceral compared to somatic pain were examined, using established pain models within the visceral and somatic fear conditioning literature, suited for individual matching of visceral and somatic pain stimuli. In this study, (Hyp.1a) shared neural responses in areas associated with sensory-discriminative pain aspects across modalities were expected. Moreover, (Hyp.1b) differential neural responses, i.e., enhanced neural responses for visceral compared to somatic pain were expected in regions linked to processing of emotional-affective pain aspects and salience processing. Lastly, the functional relation between pain-induced neural activation and anticipatory cue-induced neural responses was explored for the visceral compared to the somatic modality.

In the second study, a differential fear conditioning paradigm was implemented with visceral and somatic pain as competing US signaled by two distinct pain-predictive CS^+ compared to a non-pain-predictive CS^-. Based on the preparedness theory, it was expected that after fear conditioning, (Hyp.2a) CS^+ predicting visceral pain elicit enhanced pain-related fear responses on a behavioral and neural level compared to somatic pain-predictive CS^+ as well as to non-pain-predictive CS^-. In this regard, it was assumed that the CS-US association would be acquired more rapidly for the visceral compared to the somatic modality. Moreover, (Hyp.2b) the extinction of visceral pain-related fear responses was expected to be more fragile, i.e., more susceptible to interference by selective reinstatement, resulting in a higher reactivation for the visceral compared to the somatic modality. In a final step, the overlap of differential CS-induced neural activation across learning phases was explored.

3. Design and methods

Results from study 1 have been published (for behavioral and neural responses to pain stimuli: Koenen et al., 2018a; Koenen et al., 2017); for the functional relation between cue- and pain-induced neural responses: Koenen et al., 2018b). Results from study 2 are unpublished (currently in preparation for publication). Since study 2 was conducted consecutively after study 1 and was based on methods and results from study 1, several methodological aspects are identical (i.e., recruitment and inclusion/exclusion criteria, experimental pain models as well as thresholding, calibration and matching of visceral and somatic pain stimuli). These identical methodological aspects are therefore presented for both studies together in the following sections (3.1.-3.4.). Differences between studies are described separately in subsequent sections (study 1: 3.5.; study 2: 3.6.).

3.1. Recruitment and inclusion/exclusion criteria (studies 1 and 2)

For both studies, healthy female volunteers (study 1: N=22; study 2: N=34) were recruited by local advertisement with identical inclusion and exclusion criteria. Given the female preponderance of functional gastrointestinal disorders (Farmer et al., 2014) and the broad evidence of potential sex- and/or gender-specific differences in learning and memory processed of pain-related fear (Benson et al., 2014; Meulders et al., 2012a), modality-specific effects were examined in women in both studies. Note that participation in study 1 was exclusionary for participation in study 2. Hence, there is no overlap in study populations between studies. The recruitment process included an initial telephone screening followed by a personal interview and medical examination. All participants were aged between 18 and 45 years and had normal-ranged body mass indices (BMI) between 18 and 30 kg/m^2. Any medical condition based on self-report and any regular medication use (except thyroid medication, hormonal contraceptives, over-the-counter allergy treatment or irregular use of over-the-counter pain medication) led to exclusion of the studies. Moreover, participants were screened with a standardized questionnaire (Lacourt et al., 2014) to exclude upper or lower gastrointestinal symptoms suggestive of a functional or organic gastrointestinal condition (cut-off for exclusion: sum score $\geq$ 13/36). Clinically-relevant anxiety or

depression symptoms were exclusionary (cut-off for exclusion: sum scores ≥ 8/21), as assessed with the Hospital Anxiety and Depression Scale, (Herrmann-Lingen et al., 2007). Right-handedness was confirmed with a questionnaire on motor asymmetries (Reiss et al., 2000). Additionally, all participants were examined digitally to exclude perianal tissue damage (i.e., hemorrhoids or fissures) which may interfere with balloon placement or rectal distensions. Pregnancy was excluded with a commercially available urinary pregnancy test on the day of the experiment. The usual exclusion criteria for magnetic resonance imaging (MRI, i.e., ferro-magnetic implants, non-removable metallic devices or accessories, large tattoos) applied and structural brain abnormalities were ruled out upon structural MRI. Both study protocols were approved by the local ethics committee of the University Hospital Essen (protocol number 10-4493) and followed the Declaration of Helsinki. All participants gave informed written consent and were paid for their participation. Both studies were funded by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) within the research unit FOR 1581 "Extinction Learning. Neural Mechanisms, Behavioral Manifestations, and Clinical Implications" (FOR 1581; EL 236/9-2) and the Collaborative Research Center "Extinction Learning" (SFB 1280, Project A10).

3.2. Study protocols (overview)

Participants completed the study protocols on one day (study 1: duration 1.5 hours; study 2: duration 2.5 hours). On the study day, at first the rectal balloon was placed and the thermode device was attached to the left volar forearm. Next, individual visceral and somatic pain thresholds were assessed using well-established experimental pain models (for details see 3.3.) and a calibration and matching procedure was implemented to calibrate visceral and somatic stimuli individually-matched for perceived pain intensity (for details see 3.4.). After calibration and matching was accomplished, participants underwent a structural MRI. This was followed by an event-related functional magnetic resonance imaging (fMRI) to assess BOLD-data during the experimental phases. Moreover, subjective ratings were obtained on visual analogue scales in both studies (for details on study 1: see 3.5.; for details on study 2 see 3.6.), as well as skin conductance responses (data not reported herein).

3.3. Experimental pain models (studies 1 and 2)

Two well-established experimental pain models were used for visceral and somatic pain stimulation in both studies. For visceral pain, pressure-controlled rectal distensions were delivered with a barostat system (modified ISOBAR 3 device, G & J Electronics, Toronto, ON, Canada). Graded distensions of the rectum with an inflatable balloon system constitute an acknowledged and well-validated model used in experimental studies addressing visceroception and visceral pain (Elsenbruch et al., 2014; Mertz et al., 1995) as well as in fear conditioning studies (Gramsch et al., 2014; Icenhour et al., 2017; Icenhour et al., 2015; Labrenz et al., 2016). The distension model allows the incremental and individualized calibration of sensory and discomfort/pain thresholds and the controlled application of distensions inducing mild, intermediate, or strong sensations of urgency and pain in the context of experimental studies. These sensations are reported to closely resemble aversive visceral sensations experienced by patients with functional gastrointestinal disorders, such as IBS, as well as rectal sensations experienced by healthy persons, for instance during acute gastrointestinal infections or bloating (Roderigo et al., 2017).

For somatic pain, cutaneous heat stimuli were applied on the left ventral forearm (approximately 10cm from the wrist) with an MR-compatible thermal device (PATHWAY model CHEPS; Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel). Thermal stimulation is widely used as a validated methodological approach in experimental pain research (Fruhstorfer et al., 1976). It reliably induces slow painful sensations on the cold-heat spectrum and has been included in the highly-regarded quantitative sensory testing protocol (Rolke et al., 2006). It allows the assessment of sensory and pain thresholds, and can be utilized to quantify hyperalgesia in clinical research contexts, such as in patients with musculoskeletal chronic pain syndromes (Staud et al., 2012). Moreover, thermal heat stimuli have previously been used to examine the neural basis of impairment of learning and memory function by pain (Forkmann et al., 2016) and in pain-related fear conditioning studies (Jenewein et al., 2016).

3.4. Threshold estimation, calibration and matching of pain stimuli (studies 1 and 2)

Pain thresholds were estimated using the method of limits for the visceral (Mertz et al., 1995) and somatic modality (Fruhstorfer et al., 1976) in randomized order across participants. For visceral pain, ascending pressure pulses were applied with a duration of 30s, starting at 5mmHg. After every distension, participants were asked to rate their perception on a Likert-type scale ranging from 1 = no perception, 2 = likely perception, 3 = urge to defecate to 4 = painful perception. Pressure volumes were increased with increments set at 5mmHg until participants rated the distension as 4 = painful, which was defined as the visceral pain threshold. For safety reasons, the limit of maximal distension pressure applied was set at 55mmHg. For somatic pain, thresholds were assessed using ramped stimuli increasing from a baseline set at 32°C with 1°C/s until terminated by participants by clicking a button on a MR-compatible computer mouse by the first sensation of pain. A temperature limit was at 50°C to avoid tissue damage. This procedure was repeated five times to habituate participants to thermal stimuli and mean values of the last four stimuli were defined as somatic pain threshold. Of note, participants exceeding the maximum of 55mmHg or 49°C without reporting a feeling of any pain or discomfort led to the abortion of the experiment.

As a next step, a calibration and matching procedure was performed to identify individually-calibrated visceral and somatic pain stimuli matched for perceived pain intensity. To do so, participants were initially prompted to rate the perceived pain intensity of rectal distensions repeatedly on a digitized visual analogue scale (VAS: 0mm = “not painful at all”, 100mm = “extremely painful”), starting with a pressure value -5mmHg below pain threshold (therefore 50mmHg at maximum), to calibrate a visceral pain stimulus to a value between VAS 50 and 70. During subsequent visceral-somatic matching, heat pain stimuli were presented simultaneously to rectal distensions (30s per stimulus), with aligned durations of ascending (heating/inflation) and descending (cooling/deflation) phases of temperatures or pressures, respectively. After the stimulus presentation, participants were prompted to rate whether the heat pain stimulus was perceived as more painful, equally painful or less painful compared to the rectal distension on a Likert-type response scale. Consequently, the intensity of heat pain stimuli was adjusted by applying higher/lower temperature values with stepwise

decreasing temperature differences, (starting with ±1°C, followed by ±0.5°C, 0.3°C, 0.2°C and so on), until both stimuli were rated as equally painful in at least two consecutive trials. The resulting intensities for thermal heat and distension pressures, respectively, were then used for the repeated pain stimulation in study 1 and the fear conditioning procedure in study 2 during fMRI scanning.

3.5. Study 1

3.5.1. Experimental procedure

Behavioral and BOLD-responses were acquired in two consecutive experimental phases, i.e., a pain stimulation phase and an extinction phase. During the pain stimulation phase (Fig.4A), participants received a total of 20 pain stimuli (10 visceral, 10 somatic; duration 30s) with aligned durations of ascending/descending temperatures or pressures, respectively. Pain stimuli were each visually cued with a reinforcement schedule of 100%. For cues, two different visual geometrical symbols were used, i.e., a circle signaling visceral pain and a square signaling somatic pain or vice versa, which were counterbalanced across participants (duration jittered between 9-11s). Visceral and somatic trials were presented in an individualized, pseudorandomized order and each trial was followed by an inter-trial interval (black screen with white frame; duration jittered between 5-7s). During the subsequent extinction phase, cues were presented without pain stimulation (Fig.4B; behavioral and neural data assessed during the extinction phase are not shown herein, for details see Koenen et al., 2018b).

3.5.2. Visual analogue scales (VAS)

During the pain stimulation phase, participants were prompted to rate pain stimuli with regard to perceived pain intensity and unpleasantness to address differences in sensory-discriminative and emotional-affective pain aspects. Perceived pain intensity was rated after each trial on a digitized VAS with endpoints labeled as “not painful at all” (0mm) to “extremely painful” (100mm), which was virtually identical to the one used during calibration. Moreover, VAS-ratings of pain unpleasantness with endpoints labeled as “very pleasant” to “very unpleasant” were obtained before (PRE) and after the pain stimulation phase (POST). In addition, participants rated fear of pain, cue valence

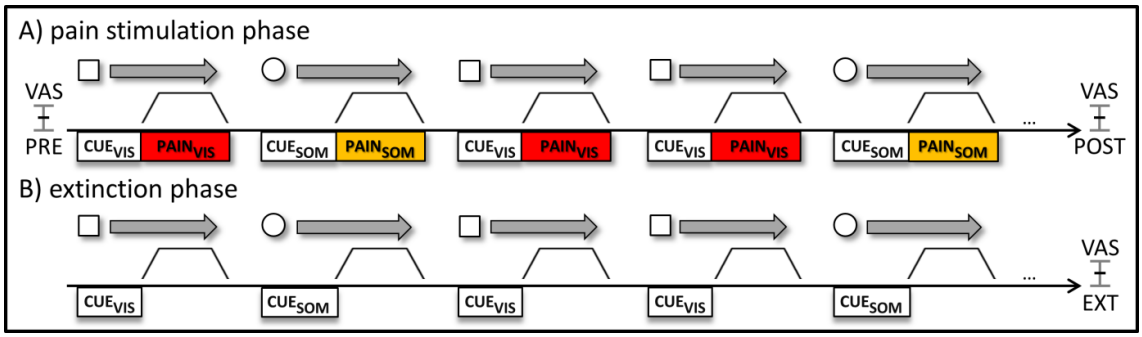

Figure 4: Experimental protocol of study 1 (N = 22). During the (A) pain stimulation phase, ten visceral and ten somatic pain stimuli were presented in pseudorandomized order, each cued by distinct visual, geometrical symbols, counterbalanced across participants (i.e., a square for visceral and a circle for somatic pain). During the (B) extinction phase, visual cues were presented without any pain stimulation in the same order as during acquisition. Before and after learning phases, participants rated fear of pain, pain unpleasantness as well as cue valence and CS-US contingency awareness on VAS-scales. Moreover, trial-by-trial VAS-ratings of perceived pain intensity were obtained. For more details, see methods section (3.5.). Abbreviations: ACQ, acquisition; EXT, extinction; PRE, pre-measurement; SOM, somatic; VAS, visual analogue scales; VIS, visceral.

and cue-pain contingency awareness before and after each experimental phase (BASE, PRE, POST). Results on these behavioral measures are reported in detail elsewhere (see Koenen et al., 2017).

3.5.3. Statistical analyses of behavioral data

For behavioral data analyses, we used the Statistical Package for the Social Sciences (SPSS, IBM Corp., IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). For analyses of pain intensity, repeated measures analysis of variance (rmANOVA) were conducted using the within-subject factors "modality" ($PAIN_{VIS}$, $PAIN_{SOM}$) and "time point" (10 trials). Individual means for trial-by-trial ratings of perceived pain intensity were calculated, in order to characterize differences between pain modalities and for implementation as covariates within BOLD data analyses (for details on analyses of trial-by-trial pain intensity ratings, please see Koenen et al., 2017). For analyses of pain unpleasantness, rmANOVA were conducted using the within-subject factors "modality" ($PAIN_{VIS}$, $PAIN_{SOM}$) and "time point" (PRE-POST). Greenhouse-Geisser correction was applied when necessary and effect sizes (eta square, η^2) are reported for rmANOVA effects. Post-hoc testing was carried out using paired t-tests, corrected for multiple comparisons (Bonferroni method). Exact p-values are

presented throughout the result section (unless $p>.99$ or $p<.001$) and effect sizes are provided (Cohen's d), corrected for correlations between variables (Dunlap et al., 1996). Results are reported as mean ± standard error of the mean (SEM).

3.5.4. FMRI data acquisition

All MR images were acquired using a whole-body 3T scanner equipped with a 32-channel head coil (Skyra, Siemens Healthcare, Erlangen, Germany). Assessment of structural images was performed using a T1-weighted 3D-MPRAGE sequence with TR 1900ms, TE 2.13ms, flip angle 9°, FOV 239 x 239mm^2, 192 slices, slice thickness 0.9mm, voxel size 0.9 x 0.9 x 0.9mm^3, matrix 256 x 256mm^2, GRAPPA r = 2. In this study, functional imaging was conducted using a multi-echo EPI sequence (Poser et al., 2006) with three echoes (echo 1 TE 13.0ms, echo 2 TE 28.9ms, echo 3 TE 44.8ms), TR 2500ms, flip angle 82°, FOV 220 x 220mm^2, matrix 80 x 80mm^2, GRAPPA r = 3 with 37 transversal slices angulated in the direction of the corpus callosum, slice thickness of 3mm, slice gap 0.6mm and a voxel size of 2.8 x 2.8 x 3.0mm.

3.5.5. FMRI data analyses

For analyses of functional images SPM 8 was used (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) as implemented in Matlab R2012a (Mathworks Inc., Sherborn, MA, USA). Initially, the three echoes of the multi-echo echo planar imaging (ME-EPI) sequence were combined, followed by slice-time and motion correction. Next, functional images were normalized using a standardized template provided by the Montreal Neurological Institute (MNI). Subsequently, smoothing with an isotropic Gaussian kernel of 8mm was applied. To correct for low frequency drifts a temporal high-pass filter with a cut-off set at 128s was implemented. Serial autocorrelations were taken into consideration by means of an autoregressive model first-order correction.

For first-level analyses of BOLD responses during the pain stimulation phase, a general linear model was applied to the EPI images, fitting the time series of each voxel with a corresponding task regressor that modeled a box car convolved with a canonical hemodynamic response function (hrf). The following regressors were included in the first-level model: visceral cues (CUE_{VIS}, 10 trials), somatic cues (CUE_{SOM}, 10 trials), visceral pain ($PAIN_{VIS}$, 10 trials) and somatic pain ($PAIN_{SOM}$, 10 trials). For analyses of

pain-induced neural responses ($PAIN_{VIS}$, $PAIN_{SOM}$), 2 separate regressors were defined for each pain stimulus: One for the ascending phase (duration of balloon inflation/thermode heating) and one for pain plateau (phase of constant balloon pressure and cutaneous heat stimulation, respectively) given previous evidence promoting differences in perception and neural activation between inflation and plateau for the visceral modality (Smith et al., 2011). For motion correction, six realignment parameters for translation (x, y, z) and rotation (pitch, roll, yaw) were implemented as multiple regressors within the model estimation, to describe the rigid body transformation between each image and a reference image. After model estimation, differential first-level contrasts were computed for neural responses to pain-predictive cues ($CUE_{VIS} > CUE_{SOM}$ and vice versa) and pain stimuli ($PAIN_{VIS} > PAIN_{SOM}$ and vice versa, each for ascending and plateau phase) during the pain stimulation phase.

On the second level, two types of conjunction analyses (Friston et al., 1999; Nichols et al., 2005) were conducted (Hyp.1a) to identify joint activations for visceral and somatic pain, using separate first-level contrasts ($PAIN_{VIS}$, $PAIN_{SOM}$). To test for shared pain-induced activation ($PAIN_{VIS}$ x $PAIN_{SOM}$) of <u>all</u> tested subjects, (a) a more conservative method against the minimum statistic to the conjunction null was conducted (Friston et al., 1999). Moreover, given the small sample size and the within-subject factor of pain-modality, (b) a more liberal approach against the minimum statistics to the global null was performed to test for shared activation in <u>some</u> subjects, as suggested by Nichols and colleagues (2005).

Moreover, differential analyses were performed (Hyp.1b) using one-sample t-tests based on differential first-level contrasts for cue-induced ($CUE_{VIS} > CUE_{SOM}$ and vice versa) and pain-induced neural responses ($PAIN_{VIS} > PAIN_{SOM}$ and vice versa). To control for individual differences between modalities in perceived pain intensity, analyses of pain-related neural activation were conducted including mean differences in pain intensity ratings as a covariate of no interest. Moreover, mean differences in pain unpleasantness, assessed after the pain stimulation phase (POST), were implemented as a covariate.

For second level analyses of shared and differential neural activation to visceral and somatic pain, regions-of-interest (ROI) were defined based on existing brain imaging

findings for pain anticipation and pain processing in the visceral and somatic pain research field (Dunckley et al., 2005; Iannetti et al., 2010; Mayer et al., 2015; Palermo et al., 2015; Strigo et al., 2003). ROI included primary and secondary somatosensory cortices (S1, S2), posterior parietal cortex (PPC), insula (anterior, posterior), thalamus, prefrontal cortex [PFC: dorsolateral (dlPFC), ventrolateral (vlPFC) and ventromedial (vmPFC) regions], anterior dorsal and perigenual (dACC, pgACC) and midcingulate cortices (MCC), amygdala, hippocampus, and dorsal pons [i.e., locus coreuleus complex (LCC)/periaqueductal gray (PAG)].

For explorative analyses of functional relations between cue- and pain-induced neural responses, multiple regression analyses were performed. To this end, percent local signal change values for differential first-level contrasts were extracted in significant ROI ($p_{FWE} < .05$) as identified in differential analyses of cue-induced neural responses ($CUE_{VIS} > CUE_{SOM}$ and vice versa). Next, the extracted parameters were implemented as independent variables (regressors) into multiple regression analyses of neural responses to visceral compared to somatic pain stimulation ($PAIN_{VIS} > PAIN_{SOM}$ and vice versa). Based on their relevance for chronic visceral pain conditions (Mayer et al., 2015; Wiech et al., 2010), key regions of the salience network (Menon et al., 2010), i.e., dorsal anterior cingulate cortex (dACC) and anterior insula were defined as ROI within these regression analyses.

For all ROI analyses, unilateral anatomical templates as implemented in SPM8 were used constructed from the WFU Pick Atlas (Version 2.5.2). To accurately differentiate functional subregions in insula (anterior, posterior) and cingulate cortex (dorsal anterior cingulate cortex (dACC), perigenual anterior cingulate cortex (pgACC) and midcingulate cortex (MCC)), masks were constructed based on existing findings (Deen et al., 2011). Additionally, a sphere was created for the localization of the periaqueductal gray (PAG), located around its anatomical center (x = 1, y = -29, z = -12; r = 10mm; Linnman et al., 2012). For the LCC, lateralized boxes were created fitted to its tube-like structure (x = (-2) – (-9)/3 – 9, y = (-36) – (-40), z = (-16) – (-36); Keren et al., 2009). Family-wise error (FWE) correction for multiple testing was set at $p_{FWE} < .05$ for all reported ROI-analyses and all coordinates refer to MNI space.

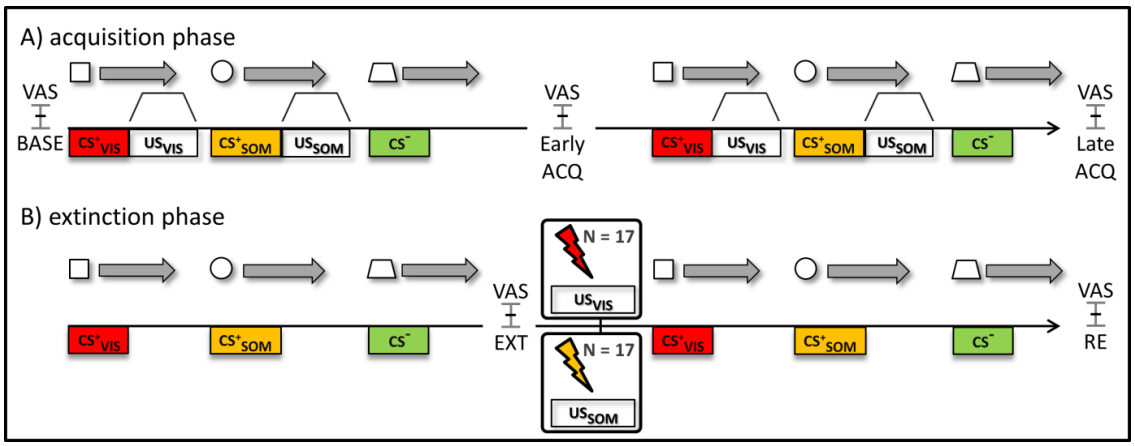

Figure 5: Experimental protocol of study 2 (N = 34). During the (A) acquisition phase, 10 CS^{+}_{VIS}, and 10 CS^{+}_{SOM} cued visceral and somatic pain stimuli, respectively, while CS^{-} were never paired with pain. Stimulus pairs were presented in pseudorandomized order with an 80% reinforcement schedule. Distinct visual, geometrical symbol were used as CS, counterbalanced across participants. During the (B) extinction phase, all CS were presented without any pain stimulation. After extinction, four unsignaled rectal distensions were applied in the visceral reinstatement group (N = 17), while the somatic reinstatement group (N = 17) received 4 unexpected heat pain stimuli: This was immediately followed by a second extinction phase in both groups without pain stimulation following CS. Before and after learning phases, participants rated CS valence, CS-US contingency awareness as well as pain unpleasantness and pain intensity on VAS-scales. For more details, see methods section (3.6.). Abbreviations: ACQ, acquisition; BASE, baseline; CS, conditioned stimulus; EXT, extinction; SOM, somatic; US, unconditioned stimulus; VAS, visual analogue scales; VIS, visceral.

3.6. Study 2

3.6.1. Experimental procedure

Behavioral and BOLD-responses were acquired in two experimental phases, i.e., an acquisition and an extinction phase. During the acquisition phase (ACQ; Fig.5A), a total of 30 visual CS (duration jittered each between 7 - 12s) were presented in individualized, pseudo-randomized order. Specifically, visceral pain-predictive CS^{+} (CS^{+}_{VIS}; 10 stimuli) signaled visceral pain (US_{VIS}; 8 stimuli) and somatic pain-predictive CS^{+} (CS^{+}_{SOM}; 10 stimuli) signaled somatic pain (US_{SOM}; 8 stimuli), which served as competing US (duration: 30s), with a reinforcement schedule of 80%. Moreover, in this study, an additional stimulus was implemented, which was never paired with pain (CS^{-}; 10 stimuli) to establish differential conditioning to a non-pain-predictive CS^{-}. For visual CS, geometrical symbols were used (i.e., a circle, a square or

a trapezoid for either CS^{+}_{VIS}, CS^{+}_{SOM} or CS^{-}, respectively, counterbalanced across participants). Each trial was followed by an inter-trial interval (black screen with white frame: duration jittered between 5 - 7s). During the following extinction phase, the same number of CS were presented in the same order, however, without any subsequent pain stimulation (CS^{+}_{VIS}, CS^{+}_{SOM}, CS^{-}; each 10 trials; durations jittered between 7 - 12s). After extinction (EXT; Fig.5B) a selective reinstatement procedure was implemented. Specifically, 4 unsignaled visceral pain stimuli were applied for visceral reinstatement in one group (N = 17), while the other group (N = 17) received 4 unexpected somatic pain stimuli for somatic reinstatement. This was immediately followed by a second phase, in which CS were presented without further pain stimulation. Groups were assigned in pseudorandomized fashion after extinction and served as mutual control groups, allowing for direct comparison of modality-specific reinstatement effects.

3.6.2. Visual analogue scales (VAS)

Analogously to study 1, participants were prompted to rate perceived pain intensity and pain unpleasantness of visceral and somatic US before, mid and after acquisition (BASE, Early ACQ, Late ACQ). Moreover, to assess CS valence, participants rated CS (CS^{+}_{VIS}; CS^{+}_{SOM}; CS^{-}) on a VAS with the endpoints “very pleasant” (0mm) to “very unpleasant” (100mm). CS valence was assessed at baseline as well as after early and late acquisition (BASE, Early ACQ, Late ACQ) to address modality-specific aspects of pain-related fear learning (Hyp.2a) as well as after early extinction and reinstatement (EXT, RE) to assess modality-specific effects on selective reinstatement within and across groups (Hyp.2b). To address CS-US contingency awareness, participants rated the probability that the visual cue (CS^{+}_{VIS}; CS^{+}_{SOM}; CS^{-}) was followed by a visceral or somatic pain stimulus (US_{VIS}; US_{SOM}), respectively, on a VAS with the endpoints “never” (0mm) to “always” (100mm). Contingency awareness was assessed after the acquisition, extinction and reinstatement phases (Late ACQ, EXT, RE), respectively.

3.6.3. Statistical analyses of behavioral data

Behavioral data were analyzed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). For analyses of pain intensity and pain unpleasantness, rmANOVA were conducted using the within-subject factors “modality” (US_{VIS}; US_{SOM}) and “time point” (BASE, Early ACQ, Late ACQ). For

analyses of CS valence ratings (Hyp.2a), rmANOVA were conducted using the within-subject factors "modality" (CS^{+}_{VIS}; CS^{+}_{SOM}; CS^{-}) and "time point" (BASE, Early ACQ, Late ACQ) for analyses of modality-specific effects on the acquisition of pain-related fear (Hyp.2a). For analyses of modality-specific effects on reinstatement of pain-related fear, a second rmANOVA was conducted with the factors "modality" (CS^{+}_{VIS}; CS^{+}_{SOM}; CS^{-}), "time point" (EXT, RE) and a between-group factor for analyses of group-wise reinstatement (RE_{VIS}-group versus RE_{SOM}-group; Hyp.2b). For analyses of CS-US contingency awareness (Hyp.2a), a rmANOVA was conducted using the within-subject factors "modality" (CS^{+}_{VIS}; CS^{+}_{SOM}; CS^{-}) and "time point" (Late ACQ, EXT, RE). Greenhouse-Geisser correction was applied when necessary and effect sizes are reported for rmANOVA effects (eta square, η^2). Post-hoc testing was accomplished using two-tailed and paired t-tests. Exact Bonferroni-corrected p-values are presented throughout the result section and effect sizes are provided (Cohen's d), analogously to study 1. Results are reported as mean ± SEM.

3.6.4. FMRI data acquisition

Technical set-up of the MRI scanner and assessment of structural fMRI data was virtually identical to study 1. However, for functional imaging, a single echo EPI sequence was used with TR 2300ms, TE 28.0ms, flip angle 90°, FOV 220 x 220mm^2, matrix 94 x 94mm^2, GRAPPA r = 2 with 38 transversal slices angulated in the direction of the corpus callosum, slice thickness of 3mm, slice gap 0.6mm and a voxel size of 2.3 x 2.3 x 3.0mm.

3.6.5. FMRI data analyses

Functional images were analyzed with SPM 12 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) implemented in Matlab R2016 (Mathworks Inc., Sherborn, MA, USA) using predefined default settings as follows. Initially, a standard realignment procedure was performed, using a smoothing kernel with 5mm FWHM followed by a slice time and motion correction. During subsequent co-registration, individual T1-weighted structural images were used as reference image, with the origin set to the anterior commissure. Next, functional images were normalized using standardized ICBM templates for European brains as implemented in SPM12 and smoothed using an isotropic Gaussian kernel of 8mm. To correct for low frequency

drifts, a temporal high-pass filter with a cut-off set at 128s was implemented. Serial autocorrelations were taken into consideration by means of an autoregressive model first-order correction.

First-level analyses were performed using a general linear model applied to the EPI images. The time series of each voxel was fitted with a corresponding task regressor that modeled a box car convolved with a canonical hemodynamic response function (hrf). In first-level analyses, BOLD-responses were analyzed for early and late acquisition (Early ACQ, Late ACQ), separately, for the extinction (EXT) and the reinstatement phase (RE). For the acquisition phase, the following regressors were included: visceral pain-predictive CS^+ (CS^+_{VIS}; 10 trials), somatic pain-predictive CS^+ (CS^+_{SOM}; 10 trials) and non-pain-predictive CS^- (CS^-; 10 trials) as well as visceral pain (US_{VIS}; 8 trials) and somatic pain stimuli (US_{SOM}; 8 trials). For the extinction and reinstatement phase, we included the same regressors to analyze CS-induced neural responses (CS^+_{VIS}, CS^+_{SOM}, CS^-; each 10 trials). Moreover, visceral and somatic pain applied in the beginning of the reinstatement phase were also included as regressors in the model, but were not part of the analyses. Six realignment parameters for translation (x, y, z) and for rotation (pitch, roll, yaw) to describe the rigid body transformation between each image and a reference image were implemented as multiple regressors within the model estimation for motion correction. After model estimation, first-level contrasts were computed for differential analyses between pain-predictive CS ($CS^+_{VIS} > CS^+_{SOM}$ and vice versa) as well as between the two CS^+ and CS^- ($CS^+_{VIS} > CS^-$ and vice versa; $CS^+_{SOM} > CS^-$ and vice versa) in all experimental phases (Early ACQ, Late ACQ, EXT, RE).

On the second level, the following t-statistics were computed to test our hypotheses: To address modality-specific effects during pain-related fear learning, one-sample t-tests were computed based on differential first-level contrasts to address the effect of modality-specific pain characteristics on the acquisition of differential pain-related fear responses (analyzed for early and late acquisition, separately; Hyp.2a) between pain-predictive ($CS^+_{VIS} > CS^+_{SOM}$ and vice versa) and compared to non-pain-predictive CS-induced neural responses ($CS^+_{VIS} > CS^-$; $CS^+_{SOM} > CS^-$ and vice versa) within the whole sample (N = 34). Similarly, one-sample t-tests and two-sample t-tests were computed based on differential first-level contrasts, to address effects of extinction and selective

reinstatement (Hyp.2b) within as well as between visceral (RE_{VIS}-group: N = 17) and somatic (RE_{SOM}-group: N = 17) reinstatement groups, respectively.

ROI were defined a priori based on existing brain imaging findings on pain anticipation and processing similar to the first study (see methods section 3.5.5.). Additional ROI were included based on fear conditioning studies using either visceral or somatic pain stimuli as US (Büchel et al., 2000; Icenhour et al., 2017; Icenhour et al., 2015; Sehlmeyer et al., 2009; Fullana et al., 2018; Haaker et al., 2014; Hermans et al., 2006). Therefore, ROI included amygdala, hippocampus, prefrontal cortices (dorsolateral/dorsomedial prefrontal cortices (dlPFC, dmPFC) and ventrolateral/ventromedial prefrontal cortices (vlPFC, vmPFC), insula (anterior, posterior), cingulate cortex, basal ganglia (pallidum, putamen, caudate nucleus), thalamus, periaqueductal grey (PAG), somatosensory cortices (S1, S2) and posterior parietal cortex (PPC). Additionally, masks were defined based on significant results in regions demonstrating differential CS-induced neural activation in the acquisition, extinction and reinstatement phases, respectively, to explore overlap with differential CS-induced neural activation across learning phases.

All ROI analyses were carried out using unilateral anatomical templates constructed from the WFU Pick Atlas (Version 2.5.2), as implemented in SPM12. Similar to study 1, we additionally differentiated functional subregions within insula and cingulate cortex (see methods section 3.5.5.). For all reported ROI-analyses, FWE-correction for multiple testing was used set at $p_{FWE} < .05$ and coordinates refer to the MNI space.

4. Results

4.1. Study 1

4.1.1. Participants

From a total sample of 28 participants, 6 participants were excluded due to technical difficulties (N = 3) and unsuccessful matching (N = 3). This resulted in a final sample of N = 22 participants with complete datasets (BMI: 21.9 ± 0.5 kg/m^2; age: 24.4 ± 0.6 years). Participants had a mean visceral pain threshold of 40.2 ± 1.5mmHg and a mean somatic pain threshold of 45.1 ± 0.6°C.

4.1.2. Pain intensity and pain unpleasantness during the pain stimulation phase

For analyses of trial-by-trial pain intensity ratings a significant main effect of modality ($F_{(1,21)}$ = 11.69; p = .003; η^2 = .36) as well as a significant interaction of time point and modality ($F_{(9,189)}$ = 4.73; p = .002; η^2 = .18) were observed. Initially, pain intensity ratings did not differ across modalities as intended by the matching procedure (PRE: $PAIN_{VIS}$: 71.57 ± 2.56mm, $PAIN_{SOM}$: 72.3 ± 2.00mm; $t_{(21)}$ = -0.36; p = .720; d = 0.25). However, due to a habituation that was only observed for somatic stimuli, mean perceived pain intensity was higher for visceral (POST: 73.71 ± 2.53mm) compared to somatic pain (POST: 61.95 ± 4.15mm; $t_{(21)}$ = -3.42; p = .003; d = 0.13).

Analyses of pain unpleasantness revealed a main effect of modality ($F_{(1,21)}$ = 30.40; p < .001; η^2 = .59) and a significant interaction of time point and modality ($F_{(1,21)}$ = 19.08; p < .001; η^2 = .48). Interestingly, visceral pain was comparable to somatic pain before (PRE: $PAIN_{VIS}$: 29.73 ± 10.95mm, $PAIN_{SOM}$: 11.45 ± 9.20mm), but perceived as more unpleasant compared to somatic pain after the pain stimulation phase (POST: $PAIN_{VIS}$: 71.91 ± 9.24mm, $PAIN_{SOM}$: -4.09 ± 12.5mm; $t_{(21)}$ = -5.59; p < .001; d = 1.46).

4.1.3. BOLD data

4.1.3.1. Shared neural responses across pain modalities

To address shared neural responses (HYP.1a) for visceral and somatic pain, conjunction analyses were performed ($PAIN_{VIS}$ x $PAIN_{SOM}$). When applying a more conservative

Table 1: Shared neural activation induced by visceral and somatic pain in study 1 (N = 22)

			MNI-coordinates					
contrast		**region**	**H**	**x**	**y**	**z**	**t-value**	**p_{FWE}**
(A) conjunction null	ascending phase	dlPFC/ anterior PFC	R	46	50	16	5.67	0.007
	pain plateau	S1	R	52	-40	44	5.01	0.011
		PPC	R	56	-40	54	5.96	<0.001
			L	-54	58	42	4.59	0.030
		vlPFC	R	48	24	-10	6.70	0.001
(B) global null	ascending phase	PPC	R	56	-40	56	3.97	<0.001
			L	-52	-58	48	4.12	<0.001
	pain plateau	dlPFC/ anterior PFC	R	48	50	10	4.79	<0.001
		vlPFC/ dlPFC	R	40	24	8	4.07	<0.001
		vmPFC	R	16	22	-16	6.70	<0.001

Results of conjunction analyses testing shared neural activation induced by visceral and somatic pain ($PAIN_{VIS}$ x $PAIN_{SOM}$), with minimum t-statistic to (A) conjunction null or (B) global null, for both pain regressors (ascending phase and pain plateau). Peak voxels indicate results of ROI analyses with a threshold at $p_{FWE}<.05$. Exact unilateral p-values are given. For more details on BOLD data analyses, see result section (4.1.3.1.). Abbreviations: dlPFC, dorsolateral prefrontal cortex; FWE, family-wise error; MNI, Montreal Neurological Institute; PFC, prefrontal cortex; PPC, posterior parietal cortex; ROI, regions of interest; S1, primary somatosensory cortex; SOM, somatic; VIS, visceral; vlPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex.

criterion, results revealed joint activations within dlPFC during the ascending phase and within S1, PPC and vlPFC during pain plateau for all participants (conjunction null; all $p_{FWE} < .05$; Tab.1A). For the more liberal analysis, significant neural activation in PPC during the ascending phase and in dlPFC, vlPFC and vmPFC during pain plateau was observed shared by some participants (global null; all $p_{FWE} < .05$; Tab.1B).

4.1.3.2. Differences in neural responses between pain modalities

In order to test differences between modalities (HYP.1b), one-sample t-tests were computed on differential first-level contrasts ($PAIN_{VIS} > PAIN_{SOM}$ and vice versa) while controlling for individual mean differences in perceived pain intensity between modalities. For somatic pain, a greater activation of vlPFC and dlPFC during the ascending phase and in posterior insula and hippocampus during pain plateau was

Table 2: Differences in neural activation between visceral and somatic pain in study 1 (N = 22)

			MNI-Coordinates					
contrast		**region**	**H**	**x**	**y**	**z**	**t-value**	**p_{FWE}**
(A) $PAIN_{VIS} < PAIN_{SOM}$	ascending phase	vlPFC	L	-52	28	26	4.51	0.032
		dlPFC	L	-50	32	2	4.60	0.027
	pain plateau	posterior insula	R	40	-14	20	4.10	0.002
			L	-40	-14	20	3.71	0.004
		HC	R	22	-6	-18	4.19	0.033
(B) $PAIN_{VIS} > PAIN_{SOM}$	ascending phase	S1	R	16	-32	50	4.44	0.015
		dACC	L	-4	8	40	4.53	0.006
		MCC	L	-6	-4	44	3.87	0.020
		anterior dorsal insula	R	40	8	4	3.68	0.006
			L	-40	4	4	4.19	0.002
		anterior ventral insula	L	-34	12	8	3.27	0.008
		PAG	R	2	-32	-12	3.72	0.005
		dorsal pons (LCC)	L/ R	0	-32	-18	4.36	0.004
	pain plateau	–	–	–	–	–	–	–

Differences between pain modalities in neural activation assessed with one sample t-tests computed on differential first level contrasts (A) $PAIN_{VIS} > PAIN_{SOM}$ and (B) $PAIN_{VIS} < PAIN_{SOM}$ during the ascending phase and the plateau phase, with mean difference in perceived pain intensity as a covariate of no interest. Peak voxels indicate results of ROI analyses with a threshold at $p_{FWE}<.05$. Exact unilateral p-values are given. For an illustration of findings, see Fig.6. For more details on BOLD data analyses, see result section (4.1.3.2.). For an illustration of findings, see Fig.6. Abbreviations: dACC, dorsal anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; FWE, family-wise error; HC; hippocampus; LCC, locus coeruleus complex; MCC, midcingulate cortex; MNI, Montreal Neurological Institute; PAG, periaqueductal gray; ROI, regions of interest; S1, primary somatosensory cortex; SOM, somatic; VIS, visceral; vlPFC, ventrolateral prefrontal cortex.

observed ($PAIN_{VIS} < PAIN_{SOM}$; all $p_{FWE} < .05$; Tab.2A; Fig.6A). Analyses revealed a greater activation of S1, dACC, MCC, dorsal and ventral anterior insula, PAG and dorsal pons (i.e., LCC) for visceral compared to somatic pain during the ascending phase ($PAIN_{VIS} > PAIN_{SOM}$; all $p_{FWE} < .05$; Tab.2B; Fig.6B). During pain plateau, this

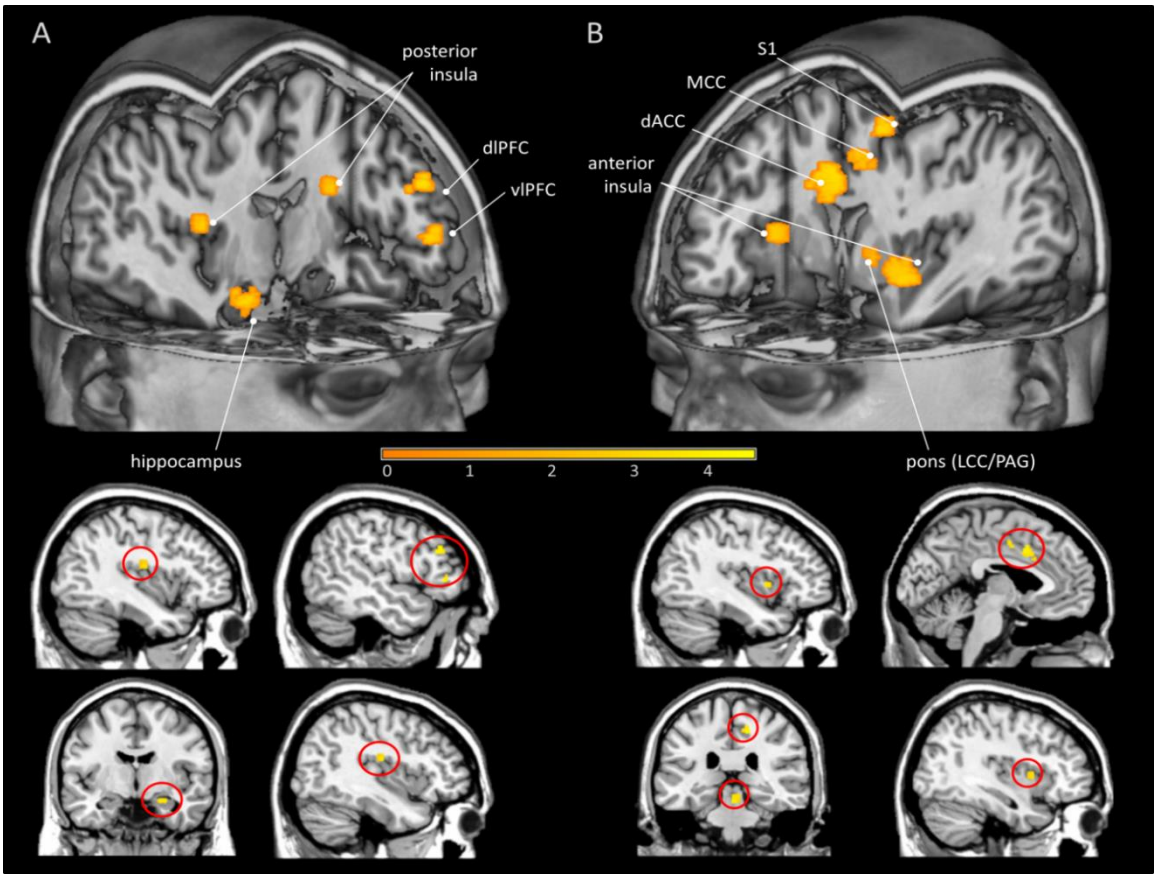

Figure 6: Differential neural responses for visceral compared to somatic pain. Enhanced neural activation was revealed in cingulate, insular, sensory-motor, hippocampal, prefrontal and midbrain regions for (A) somatic pain [$PAIN_{VIS}$ < $PAIN_{SOM}$] and (B) visceral pain [$PAIN_{VIS}$ > $PAIN_{SOM}$] combined for the ascending and plateau phase of pain stimulation [in all contrasts: p_{FWE} < .05; for details, see Tab.3]. Activations were superimposed on a structural T1-weighted MRI used for spatial normalization and thresholded at p < .001 uncorrected for visualization purposes. Color bars indicate t-scores. For more details on BOLD data analyses, see Tab.2 and result section (4.1.3.2.). Abbreviations: dACC, dorsal anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; LCC, locus coeruleus complex; MCC, midcingulate cortex; PAG, periaqueductal gray; S1, primary somatosensory cortex; vlPFC, ventrolateral prefrontal cortex.

contrast revealed no significant effects. Additional inclusion of mean differences in pain unpleasantness as a covariate did not alter the results.

4.1.3.3. Functional relation between cue- and pain-induced neural responses

Analyses of differential cue-induced neural responses (CUE_{VIS} > CUE_{SOM} and vice versa) revealed a significantly enhanced deactivation to visceral compared to somatic cues in the posterior insula. To estimate the functional relation between cue- and pain-

Table 3: Functional relation of modality-specific cue-induced neural activation in the posterior insula to modality-specific pain-induced neural responses in study 1 (N = 22)

regressors	left posterior insula (x = -32, y = -20, z = 16)						right posterior insula (x = 42, y = -18, z = 20)					
	MNI-coordinates						MNI-coordinates					
region	H	x	y	z	t-value	p_{FWE}	H	x	y	z	t-value	p_{FWE}
anterior insula	R	–	–	–	–	–	R	32	12	-4	3.39	0.007
	L	-36	4	0	2.84	0.032	L	-30	14	10	3.36	0.008
dACC	R	–	–	–	–	–	R	6	12	34	4.95	0.004
	L	-2	12	32	4.20	0.014	L	-2	12	32	4.65	0.006

Results from multiple regression analyses as implemented in SPM. Percent local signal change values for differential first-level contrasts were extracted in peak voxels of right and left posterior insula during pain anticipation. Next, the extracted parameters were implemented as independent variables within analyses of pain-induced neural BOLD responses ($PAIN_{VIS}$ > $PAIN_{SOM}$ and vice versa). Peak voxels indicate results of ROI analyses with a threshold at pFWE<.05 and exact unilateral p-values are given. For more details on BOLD data analyses, see result section (4.1.3.3.). For an illustration of findings, see Fig.7. Abbreviations: dACC, dorsal anterior cingulate cortex; FWE, family-wise error; MNI, Montreal Neurological Institute; ROI, regions of interest; SOM, somatic; VIS, visceral.

induced neural responses, percent local signal changes of bilateral posterior insula peak-voxel activation were extracted during visceral and somatic cue presentation, respectively. Next, mean differences in percent signal changes were calculated and implemented as regressors in an exploratory multiple regression analyses of pain-induced neural responses in bilateral regions of the salience network (dACC, anterior insula). Interestingly, we observed that enhanced cue-induced posterior insula deactivation for the visceral compared to somatic modality was significantly correlated with enhanced differential activation in dACC and anterior insula for visceral compared to somatic pain (Tab.3; Fig.7).

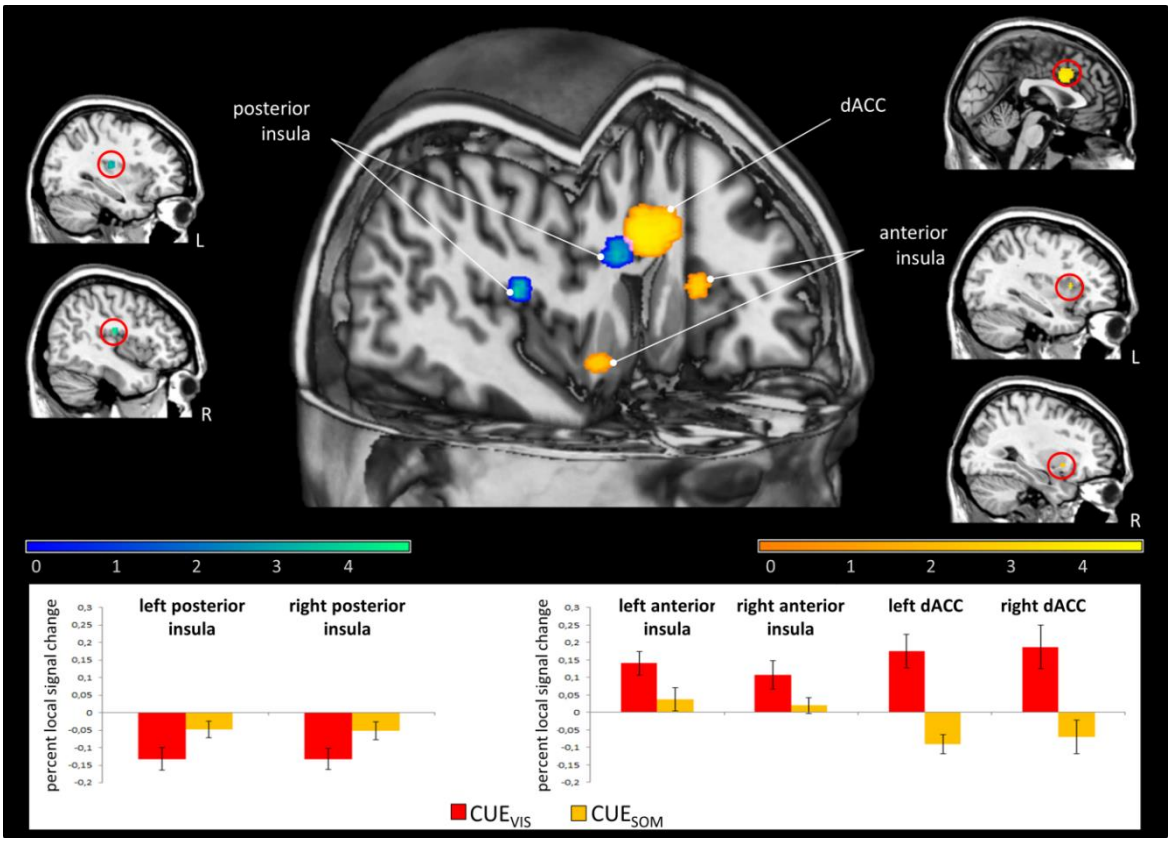

Figure 7: Functional relation between cue- and pain-induced neural responses. Differences in cue-induced neural responses during pain anticipation [$CUE_{VIS} < CUE_{SOM}$: blue-green] in the posterior insula significantly predict pain-induced neural responses [$PAIN_{VIS} > PAIN_{SOM:}$ yellow-orange] in key nodes of the salience network [in all contrasts: $p_{FWE} < .05$]. Bottom panel depicts percent local signal changes during (left) pain anticipation and (right) pain processing. For more details on BOLD data analyses, see Tab.3 and result section (4.1.3.3.). Activations were superimposed on a structural T1-weighted MRI used for spatial normalization and thresholded at $p < .001$ uncorrected for visualization purposes. Color bars indicate t-scores. Abbreviations: dACC, dorsal anterior cingulate cortex; SOM, somatic; VIS, visceral.

4.2. Study 2

4.2.1. Participants

From the total sample of 43 participants, 9 participants had to be excluded from further analyses, due to technical difficulties leading to incomplete sets of fMRI data (N = 6) or behavioral data (N = 2) or due to insensitivity to the visceral pain stimulus (N = 1). This resulted in a final data set of N = 34 participants (BMI: 22.9 ± 0.5 kg/m^2; age: 30.5 ± 1.9 years; see Fig.5A). Participants had a mean visceral pain threshold of 39.6 ± 1.9mmHg and a somatic pain threshold of 44.7 ± 0.5°C. After extinction, participants were pseudo-randomly assigned into two groups (see Fig.5B), i.e., one group that underwent a visceral reinstatement (RE_{VIS}-group: N = 17), and another group that underwent a somatic reinstatement (RE_{SOM}-group: N = 17). Reinstatement groups did not differ in age (RE_{VIS}-group: 30 ± 2.5 years, RE_{SOM}-group: age: 31 ± 3.1 years; p = .80; d = 0.09) or BMI (RE_{VIS}-group: 23.3 ± 0.8 kg/m^2, RE_{SOM}-group: 22.6 ± 0.5 kg/m^2; p = .45; d = 0.25) and had comparable visceral (RE_{VIS}-group: 39.1 ± 3.2mmHg, RE_{SOM}-group: 40.0 ± 2.0mmHg; p = .82; d = 0.08) and somatic pain thresholds (RE_{VIS}-group: 44.5 ± 0.5°C, RE_{SOM}-group: 45.0 ± 0.8°C; p = .56; d = 0.19).

4.2.2. Pain intensity and pain unpleasantness during the acquisition phase

Analyses of pain intensity revealed a main effect of modality ($F_{(1,33)}$ = 15.54; p < .001; η^2 = .32) as well as a significant interaction of time point and modality ($F_{(2,66)}$ = 15.14; p < .001; η^2 = .32). As intended by the matching procedure, pain intensity ratings were virtually equal for both modalities prior to acquisition (BASE: US_{VIS}: 70.15 ± 1.00mm, US_{SOM}: 69.85 ± 0.86mm; p > .99; d = 0.04) as well as after early acquisition (Early ACQ: US_{VIS}: 70.18 ± 1.20mm, US_{SOM}: 70.50 ± 0.86mm; p > .99; d = 0.08). However, after late acquisition, perceived pain intensity was higher for the visceral compared to the somatic pain modality (Late ACQ: US_{VIS}: 77.21 ± 1.68mm, US_{SOM}: 65.06 ± 2.97mm; p < .001; d = 0.33).

Analyses of pain unpleasantness revealed a main effect of time point ($F_{(2,66)}$ = 15.77; p < .001; η^2 = .32), modality ($F_{(1,33)}$ = 64.34; p < .001; η^2 = .58) and a significant interaction between time point and modality ($F_{(2,66)}$ = 8.52; p = .001; η^2 = .21). Visceral pain was perceived as more unpleasant compared to somatic pain before (BASE: US_{VIS}: 25.88 ±

6.69mm, US_{SOM}: 3.24 ± 4.86mm; $t_{(33)}$ = 3.85; p = .002; d = 0.44), after early (Early ACQ: US_{VIS}: 52.00 ± 3.85mm, US_{SOM}: 11.82 ± 4.95mm; $t_{(33)}$ = 6.92; p < .001; d = 0.82) and after late acquisition (Late ACQ: US_{VIS}: 66.47 ± 4.28mm, US_{SOM}: 20.24 ± 6.10mm; $t_{(33)}$ = 6.38; p < .001; d = 1.09).

Notably, mean values of visceral (RE_{VIS}-group: 72.44 ± 8.04mm, RE_{SOM}-group: 72.55 ± 7.14mm) and somatic pain intensity ratings (RE_{VIS}-group: 69.08 ± 8.67mm, RE_{SOM}-group: 67.86 ± 10.67mm; both p > .66; mean d = 0.01) were not significantly different across reinstatement groups. Moreover, no significant differences in visceral (US_{VIS}: RE_{VIS}-group: 48.51 ± 29.86mm, RE_{SOM}-group: 47.73 ± 27.05mm) and somatic pain unpleasantness (US_{SOM}: RE_{VIS}-group: 4.94 ± 28.16mm, RE_{SOM}-group: 18.59 ± 32.45mm; both p > .31; mean d = 0.23) across reinstatement groups were observed.

4.2.3. CS valence

4.2.3.1. Modality-specific effects in acquisition of pain-related fear

Analyses of CS valence revealed a main effect of time point ($F_{(2,66)}$ = 23.14; p < .001; η^2 = .41), modality ($F_{(2,66)}$ = 14.83; p < .001; η^2 = .31) and a significant interaction of time point and modality ($F_{(4,132)}$ = 17.62; p < .001; η^2 = .35). Post-hoc tests revealed that prior to acquisition, CS were all rated as equally neutral (BASE: all p > .99; mean d = 0.12). After acquisition, (Hyp.2a) CS^+_{VIS} were rated as significantly more unpleasant compared to CS^+_{SOM} (Early ACQ: $t_{(33)}$ = 4.47; p = .001; d = 0.08; Late ACQ: $t_{(33)}$ = 4.36; p = .002; d = 0.08). Moreover, CS^+_{VIS} were also rated as significantly more unpleasant compared to CS^- (Early ACQ: $t_{(33)}$ = 5.44; p < .001; d = 0.15; Late ACQ: $t_{(33)}$ = 5.52; p < .001; d = 0.17; Fig.8A-a). This was due to increasing negative valence of CS^+_{VIS} from baseline to early acquisition (BASE-Early ACQ: $t_{(33)}$ = -6.42; p < .001; d = 0.08), but not from early to late acquisition (Early-Late ACQ: p > .99; d = 0.04). Valence ratings of CS^+_{SOM} and CS^- remained virtually unchanged during acquisition (Base-Early-Late ACQ: all p > .44; d = 0.17). Consequently, there were no detectable differences in ratings of CS valence between CS^+_{SOM} and CS^- after early (Early ACQ: p > .99; d = 0.35) or late acquisition (Late ACQ: p > .56; d = 0.41; Fig.8A-a).

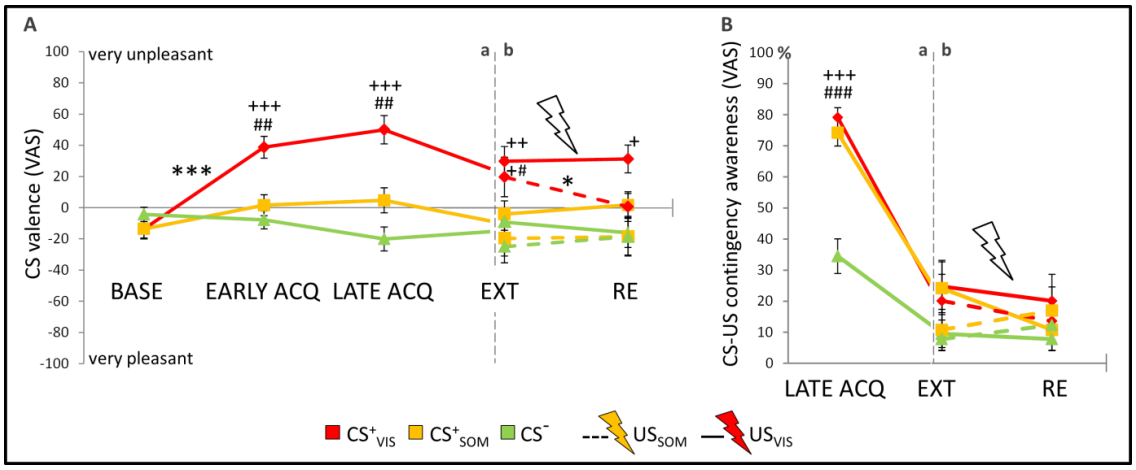

Figure 8: VAS-ratings. Ratings of (A) CS valence and (B) CS-US contingency awareness during each (a) acquisition phase and (b) extinction phases. For more details on ANOVA results, see result section (4.2.3. and 4.2.4.). Results of post-hoc comparisons: * $p < .05$; ***$p < .001$ (for changes in CS-valence); $^{+}p < .05$; $^{++}p < .01$; $^{+++}p < .001$ (for differences between CS^{+}_{VIS} and CS^{-}); $^{\#}p < .05$; $^{\#\#}p < .01$; $^{\#\#\#}p < .001$ (for differences between CS^{+}_{VIS} and CS^{+}_{SOM}). All results are Bonferroni-corrected for multiple testing. Abbreviations: ACQ, acquisition; CS, conditioned stimulus; EXT, extinction; SOM, somatic; RE, reinstatement; US, unconditioned stimulus; VAS, visual analogue scale; VIS, visceral.

4.2.3.2. Modality-specific effects in extinction and reinstatement of pain-related fear

In order to examine the effects of modality-specific reinstatement, a second rmANOVA for CS valence (EXT, RE) was conducted, including reinstatement groups (RE_{VIS}-group, RE_{SOM}-group) as a group factor. This analysis revealed a significant effect of modality ($F_{(2,64)} = 10.60$; $p < .001$; $\eta^2 = .25$) and a significant three-way interaction between modality, time point and reinstatement group ($F_{(2,64)} = 3.86$; $p = .035$; $\eta^2 = .11$).

After extinction, post-hoc tests revealed comparably low CS valence ratings between groups (EXT: RE_{VIS}-group > RE_{SOM}-group: CS^{+}_{VIS}: $p > .99$; $d = 0.23$; CS^{+}_{SOM}: $p = .79$; $d = 0.39$; CS^{-}: $p = .71$; $d = 0.89$; Fig.8A-b). Differences between ratings of CS^{+}_{VIS} and CS^{+}_{SOM} were only observed within the somatic reinstatement group (EXT: RE_{SOM}-group: $t_{(16)} = 2.83$; $p = .036$; $d = 0.07$), but failed to reach significance in the visceral reinstatement group after Bonferroni correction was applied (EXT: RE_{VIS}-group: $p = .10$; $d = 0.10$). Within both groups, CS^{+}_{VIS} valence was still significantly greater compared to CS^{-} (EXT: RE_{VIS}-group: $t_{(16)} = 3.75$; $p = .006$; $d = 0.13$; RE_{SOM}-group: $t_{(16)} = 2.80$; $p = .039$; $d = 0.15$; Fig.8A-b). However, no significant differences between valence ratings of CS^{+}_{SOM} and CS^{-} valence were detectable (EXT: $p > .99$; mean $d = 0.36$).

After reinstatement, no group differences were observed in CS valence ratings after Bonferroni correction was applied (RE_{VIS}-group > RE_{SOM}-group: CS^{+}_{VIS}: p = .06; d = 0.83; CS^{+}_{SOM}: p = .46; d = 0.50; CS^{-}: p > .99; d = 0.06; Fig8A-b). Notably, in the visceral reinstatement group, CS^{+}_{VIS} remained more unpleasant compared to CS^{-} (RE: $t_{(16)}$ = 3.71; p = .006; d = 0.17), but failed to reach significance compared to CS^{+}_{SOM} (RE: p = .11; d = 0.10). In the somatic reinstatement group, no significant differences between valence ratings of CS^{+}_{VIS} compared to CS^{-} (RE: p = .57; d = 0.15) or CS^{+}_{SOM} (RE: p = .27; d = 0.06) were observed. Consequently, CS^{+}_{VIS} valence ratings returned to baseline after somatic reinstatement (EXT-RE: $t_{(16)}$ = 3.31; p = .012; d = 0.03; Fig.8A-b), but not after visceral reinstatement (EXT-RE: p > .99; d = 0.04; Hyp.2b). Within both reinstatement groups, CS valence of CS^{+}_{SOM} and CS^{-} remained unchanged (EXT-RE: all p > .41; mean d = 0.13) and hence no differences in valence ratings were detectable between CS^{+}_{SOM} and CS^{-} (RE: RE_{VIS}-group: p = .65; d = 0.48; RE_{SOM}-group: p > .99; d = 0.29).

4.2.4. CS-US contingency awareness

Analyses of CS-US contingency awareness revealed a significant main effect of time point ($F_{(2,66)}$ = 90.83; p < .001; η^2 = .73), modality ($F_{(2,66)}$ = 26.89; p < .001; η^2 = .45) and a significant interaction of time point and modality ($F_{(4,132)}$ = 13.89; p < .001; η^2 = .30). As intended by the 80% reinforcement schedule applied, CS-US contingencies were estimated accurately for CS^{+}_{VIS} and CS^{+}_{SOM} compared to CS^{-} after acquisition (Late ACQ: p < .001; d = 0.33) and were comparable for both modalities (Late ACQ: p > .99; d = 0.08; Fig.8B-a). Moreover, CS-US contingency ratings were also accurate and virtually identical for all CS after extinction (EXT: all p > .99 d = 0.16; Fig.8B-b) and after reinstatement (RE: all p > .99; d = 0.24; Fig.8B-b). Notably, no group differences were observed in CS-US-contingency after reinstatement (RE: RE_{VIS}-group > RE_{SOM}-group: all p>.99; d = 0.23).

4.2.5. BOLD data

4.2.5.1. Modality-specific effects in acquisition of pain-related fear

During early acquisition (Hyp.2a), enhanced deactivation for the CS^{+}_{VIS} compared to the CS^{+}_{SOM} was observed in anterior and posterior insula, MCC, PCC, primary

somatosensory and motor cortices (S1/M1; CS^+_{VIS} < CS^+_{SOM}; Fig.9A). For CS^+_{SOM} compared to the CS^-, enhanced deactivation was detectable within the dmPFC (CS^+_{SOM} < CS^-; Fig.9B). CS^+_{VIS} compared to CS^- revealed deactivation in similar regions, namely anterior and posterior insula, MCC, the thalamus and S1/M1 (CS^+_{VIS} < CS^-; Tab.4; Fig.9C). During late acquisition, decreased activation was revealed for visceral CS^+ compared to CS^- (CS^+_{VIS} < CS^-: L: x = -10; y = -28; z = 68; t = 5.39; p_{FWE} = .001; R: x = 20, y = -24, z = 78; t = 5.83; p_{FWE} = .001) as well as compared to CS^+_{SOM} (CS^+_{VIS} < CS^+_{SOM}: R: x= 14; y = -28; z = 72; t = 4.57; p_{FWE} = .015) in S1/M1.

Table 4: Neural responses to conditioned stimuli (CS) during acquisition in study 2 (N = 34)

		MNI-coordinates					
contrast	**region**	**H**	**x**	**y**	**z**	**t-value**	**p_{FWE}**
CS^+_{VIS} < CS^+_{SOM}	anterior insula	L	-34	2	18	4.65	0.010
		R	36	2	14	4.44	0.016
	posterior insula	L	-32	-22	22	4.20	0.030
		R	32	-22	14	4.47	0.015
	MCC	L	-6	0	36	4.66	0.009
		R	6	0	40	4.24	0.029
	PCC	L	-6	-40	6	3.87	0.018
	S1/M1	R	8	-28	76	4.31	0.010
CS^+_{VIS} > CS^+_{SOM}	-	-	-	-	-	-	-
CS^+_{VIS} < CS^-	anterior insula	L	-34	2	16	5.08	0.003
		R	34	-16	12	4.64	0.009
	posterior insula	L	-32	-22	22	4.93	0.005
		R	34	-16	12	4.64	0.009
	MCC	L	-4	-10	40	5.37	0.001
		R	4	0	30	4.57	0.012
	thalamus	L	-18	-24	-2	4.24	0.013
		R	14	-18	0	5.30	0.001
	S1/M1	R	14	-36	76	5.72	0.001
CS^+_{VIS} > CS^-	-	-	-	-	-	-	-
CS^+_{SOM} < CS^-	dmPFC	R	10	4	46	4.54	0.017
CS^+_{SOM} > CS-	-	-	-	-	-	-	-

Analyses of differential neural responses to CS^+_{VIS}, CS^+_{SOM}, and CS- during early acquisition. Peak voxels indicate results of ROI analyses with a threshold at p_{FWE}<.05 (see Fig.9) and exact unilateral p-values are given. For more details on BOLD data analyses, see result section (4.2.5.1.). For an illustration of findings, see Fig.9. Abbreviations: dmPFC, dorsomedial prefrontal cortex; FWE, family-wise error; M1, primary motor cortex; MCC, midcingulate cortex; MNI, Montreal Neurological Institute; PCC, posterior cingulate cortex; ROI, regions of interest; S1, primary somatosensory cortex; SOM, somatic; VIS, visceral.

No other significant differences in neural activation between CS were detectable during early or late acquisition. Inclusion of pain intensity or pain unpleasantness in analyses of BOLD responses during early or late acquisition as a covariate revealed no significant correlation.

4.2.5.2. Modality-specific effects in extinction and reinstatement of pain-related fear

During extinction, small clusters of enhanced activation were observed within the left putamen to CS^+_{VIS} compared to CS^- ($CS^+_{VIS} > CS^-$: x = -26; y = 12; z = 2; t = 3.89; p = .033), whereas for CS^+_{SOM} compared to CS^- enhanced neural responses in the right caudate nucleus were detectable ($CS^+_{SOM} > CS^-$: x = 14; y = 2; z = 22; t = 4.52; p = .009). No other differences in neural activation between CS were detectable.

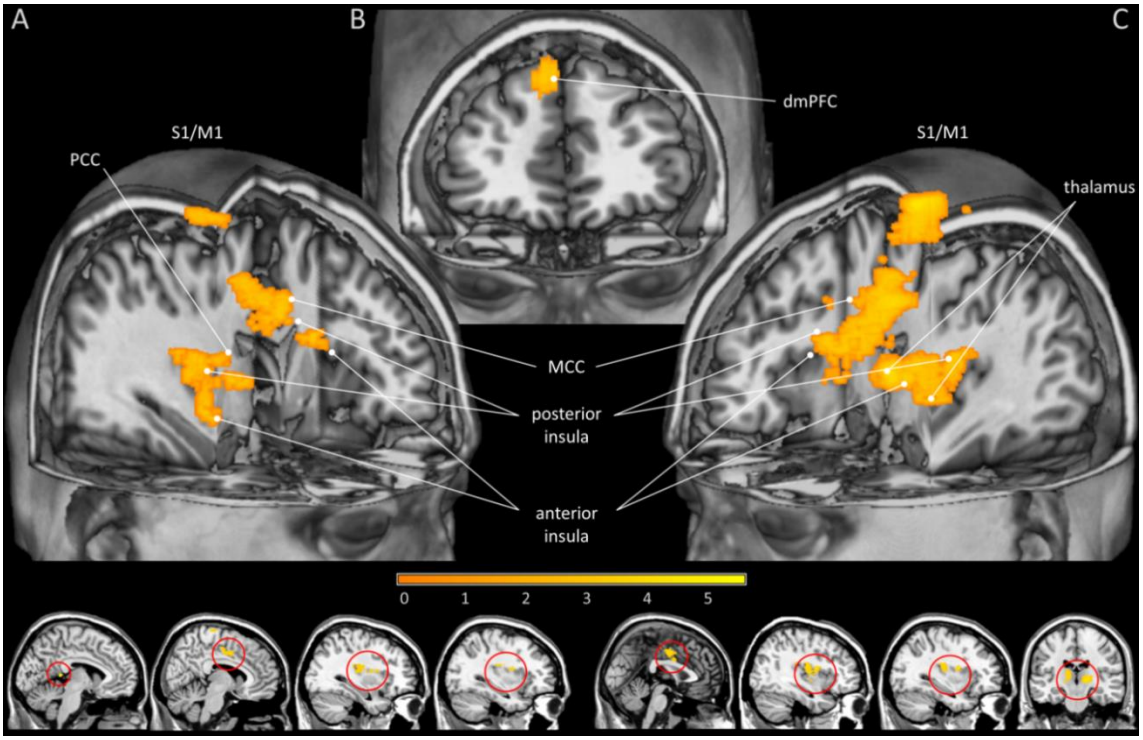

Figure 9: Differences in neural responses to conditioned stimuli during early acquisition. Differential CS-induced neural responses in a priori defined ROI in cingulate, insular, sensory-motor, thalamic and prefrontal regions between (A) pain-predicitive CS^+ [$CS^+_{VIS} < CS^+_{SOM}$], between (B) somatic [$CS^+_{SOM} < CS^-$], and (C) visceral pain-predictive CS^+ compared to the CS^- [$CS^+_{VIS} < CS^-$] [in all contrasts: $p_{FWE} < .05$]. For more details on BOLD data analyses, see Tab.4 and result section (4.2.5.1.) Activations were superimposed on a structural T1-weighted MRI used for spatial normalization and thresholded at p < .001 uncorrected for visualization purposes. Color bars indicate t-scores. Abbreviations: CS, conditioned stimuli; dmPFC, dorsomedial prefrontal cortex; M1, primary motor cortex; MCC, midcingulate cortex; PCC, posterior cingulate cortex; S1, primary somatosensory cortex.

After reinstatement, between-group analyses revealed enhanced deactivation for the CS^{+}_{VIS} compared to the CS^{+}_{SOM} in the posterior insula (left: x = -32; y = -4; z = 16; t = 4.66; p = .010; right: x = 32; y = -24; z = 22; t = 4.98; p = .004) and the thalamus (left: x = -16; y = -20; z = 8; t = 4.25; p = .013; right: x = 12; y = -24; z = -2; t = 3.78; p = .035) for the visceral compared to the somatic reinstatement group. No other significant differences in CS-induced neural activation across reinstatement groups were detectable.

Within-group analyses revealed significantly enhanced deactivation to CS^{+}_{VIS} compared to CS^{+}_{SOM} in anterior und posterior insula, ACC, MCC, hippocampus and amygdala in the visceral reinstatement group (Hyp.2b; Tab.5A). However, no other significant

Table 5: Neural responses to conditioned stimuli (CS) after reinstatement within the visceral (N=17) and the somatic reinstatement group (N=17) in study 2

			MNI-coordinates					
contrast		**region**	**H**	**x**	**y**	**z**	**t-value**	**p_{FWE}**
A) RE_{VIS}-group	$CS^{+}_{VIS} > CS^{-}$	-	-	-	-	-	-	-
	$CS^{+}_{VIS} < CS^{-}$	-	-	-	-	-	-	-
	$CS^{+}_{VIS} < CS^{+}_{SOM}$	anterior insula	L	-26	28	6	6.76	0.002
			R	40	-2	10	5.15	0.025
		posterior insula	L	-36	-8	12	6.27	0.005
			R	32	-16	18	5.54	0.014
		ACC	L	-6	4	30	6.09	0.005
			R	2	4	28	5.53	0.010
		MCC	R	14	-10	48	6.62	0.003
		HC	L	-32	-26	-16	5.45	0.008
			R	36	-12	-26	6.14	0.003
		amygdala	L	-24	-4	-20	4.00	0.023
	$CS^{+}_{VIS} > CS^{+}_{SOM}$	-	-	-	-	-	-	-
	$CS^{+}_{SOM} > CS^{-}$	-	-	-	-	-	-	-
	$CS^{+}_{SOM} < CS^{-}$	-	-	-	-	-	-	-
B) RE_{SOM}-group	– no significant results –							

Analyses of differential activation for CS^{+}_{VIS}, CS^{+}_{SOM}, and CS- following (A) visceral or (B) somatic reinstatement (both groups: N = 17). Peak voxels indicate results of ROI analyses with a threshold at $p_{FWE}<.05$ and exact unilateral p-values are given. For more details on BOLD data analyses, see result section (4.2.5.2.). Abbreviations: ACC, anterior cingulate cortex; FWE, family-wise error; HC, hippocampus; MCC, midcingulate cortex; MNI, Montreal Neurological Institute; ROI, regions of interest; SOM, somatic; VIS, visceral.

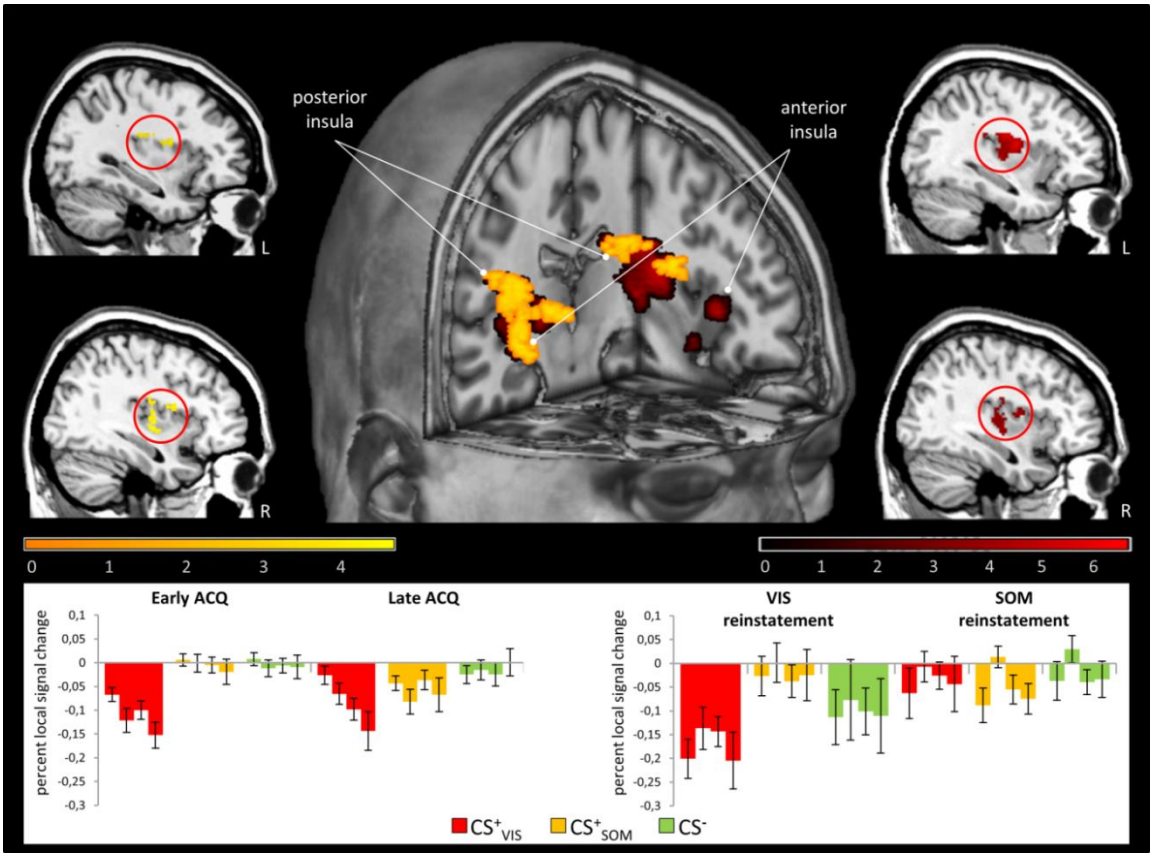

Figure 10: Overlap in the insula for CS^{+}_{VIS} compared to CS^{+}_{SOM} in different learning phases. Differential neural activation during early acquisition (yellow-orange) and after visceral reinstatement (dark red-light red) in anterior and posterior insular regions for the visceral compared to the somatic pain-predictive CS [all contrasts: $p_{FWE} < .05$]. Bottom panel depicts percent local signal changes during (left) acquisition and (right) after reinstatement. For more details on BOLD data analyses, see Tab.5 and result section (4.2.5.2. und 4.2.5.3.). Activations were superimposed on a structural T1-weighted MRI used for spatial normalization and thresholded at p<.001 uncorrected for visualization purposes. Color bars indicate t-scores. Abbreviations: ACQ, acquisition; CS, conditioned stimuli; FWE, family-wise error; SOM, somatic; VIS, visceral.

differential neural responses to CS were observed in the visceral reinstatement group. After somatic reinstatement (Tab.5B), no differences in neural activation to CS were detectable.

4.2.5.3. Functional and structural relation between learned and reactivated fear responses

Additionally, the structural overlap between insular areas showing a significant differential neural response during acquisition and after visceral reinstatement to CS^{+}_{VIS}

compared to CS^{+}_{SOM} was explored. To do so, masks generated from the results obtained in the acquisition phase were used for ROI analyses after visceral reinstatement. These analyses revealed an overlap between CS-induced activation within anterior and posterior insula during acquisition and after visceral reinstatement (Fig.10).

5. Discussion

In this thesis, two consecutive studies were accomplished in order to address the contribution of modality-specific pain characteristics on learning and extinction of pain-related fear. In the first study, joint and differential neural responses to visceral compared to somatic pain were examined at stimulus intensities that were individually-calibrated and matched to perceived pain intensity using well-established experimental pain models. Moreover, the functional relation between pain-induced neural responses and neural activation during presentation of pain-predictive cues was explored. Based on the results of this experiment, a second study was conducted. Herein, a differential delayed fear conditioning paradigm was used, addressing the role of pain modality in learning and extinction of pain-related fear using visceral and somatic pain stimuli as competing US. Moreover, the return of visceral compared to somatic pain-related fear responses was tested using a selective reinstatement procedure. The respective findings of both studies and potential clinical implications are discussed in the following sections with a focus on a priori defined hypotheses.

5.1. Neural responses in the salience network differ across pain modalities

In the first study, neural responses to visceral compared to somatic pain were examined, expecting (Hyp.1a) shared neural responses in areas associated with sensory-discriminative pain aspects and (Hyp.1b) enhanced neural responses for visceral compared to somatic pain in regions linked to emotional-affective and salience aspects of pain processing. Lastly, the functional relation between neural pain-induced neural activation and anticipatory cue-induced neural responses was explored.

The first study led to two main findings: First, a broad neural network was identified, showing joint neural activation for visceral and somatic pain in the somatosensory cortex, the PPC and several prefrontal regions, encompassing the dorsolateral, ventrolateral and ventromedial prefrontal cortex. In line with the first hypothesis (Hyp.1a), joint neural responses were observed in areas associated with processing of sensory-discriminative pain aspects (S1), and areas of the fronto-parietal attention network (PPC, dlPFC, vlPFC). These findings suggest similar perceptual and cognitive

processes, such as appraisal, as well as reflexive attentional processes to painful stimuli independent of modality (Ptak, 2012), in line with the notion that pain stimuli are inherently salient stimuli that require immediate action, irrespective of pain modality. The detected brain areas also correspond to neural networks which are commonly (Moisset et al., 2007), but not exclusively (Iannetti et al., 2010), associated with the "pain matrix". Given that both visceral and somatic pain stimuli were aversive and painful, the shared activation in these areas presumably represents neural pain processing independent of modality-specific pain characteristics.

In addition to this broad shared neural network, there were also fundamental differences in behavioral and neural responses to visceral compared to somatic pain, confirming the second hypothesis (Hyp.1b). Specifically, enhanced perception of emotional-affective pain aspects (i.e., unpleasantness) for the visceral modality was complemented by enhanced neural activation observed in somatosensory and prefrontal (vlPFC, dlPFC) areas as well as insular (anterior insula) and cingulate regions (dACC, MCC) for visceral compared to somatic pain. These results substantiate and complement previous brain imaging findings on differential processing for the visceral compared to the somatic modality (Aziz et al., 2000; Dunckley et al., 2005; Strigo et al., 2003). The anterior insula together with the dACC are considered to be engaged in salience processing (Iannetti et al., 2010; Mayer et al., 2015; Menon et al., 2010; Wiech et al., 2010), but are also involved in different processes, such as emotion regulation (Langner et al., 2018), or self-awareness (Craig, 2009). Moreover, visceral pain induced enhanced activation in areas associated with descending pain modulation in the brainstem (PAG/dorsal pons). Notably, enhanced neural activation was most pronounced during the ascending phase (inflation/heating stage of stimulation) for visceral compared to somatic pain, while enhanced neural responses to somatic pain in posterior insula and hippocampus were strongest during the plateau phase. This temporal difference in stimulus integration potentially reflects a more rapid detection and evaluation of visceral compared to somatic pain stimuli.

Together, these findings support the notion that pain perception and pain processing are at least in part specific to pain modality. In addition to accumulating evidence supporting distinct psychophysiological principles underlying pain processing in different modalities (Cervero, 2009), visceral pain may be processed differently also on

supraspinal levels, suggesting a (re-)evaluation of nociceptive input based on its origin, as well as altered engagement of regions involved in descending pain modulation. The detection of interoceptive stimuli in viscerally innervated areas supposedly leads to preferential processing compared to exteroceptive, somatic pain applied to upper extremities, including enhanced involvement of attentional and emotional neural resources. In this context, the anterior insula and ACC as key nodes of the salience network (Menon et al., 2010; Wiech et al., 2010) seem to play a role in the identification and evaluation process of visceral pain stimuli, overall implying a higher biological significance of interoceptive, visceral compared to exteroceptive somatic pain. Interestingly, additional analyses suggested that anticipatory responses to visceral pain were reflected by greater deactivation within the posterior insula, which correlated with greater engagement of the salience network during pain stimulation for the visceral compared to the somatic modality. Albeit explorative, these findings suggest distinct roles of anterior and posterior parts of the insula in anticipation and processing of modality-specific pain aspects, which will be discussed in detail later (see 5.3.).

5.2. Learning and extinction of pain-related fear are shaped by pain modality

In the second study, visceral and somatic pain were implemented as competing US in a differential fear conditioning paradigm in order to examine differences in learning, extinction and reactivation of pain-related fear across pain modalities. Based on the concept of preparedness, (Hyp.2a) CS^+ predicting visceral pain were expected to elicit enhanced behavioral and neural pain-related fear responses compared to somatic pain-predictive CS^+ and to a non-pain-predictive CS^- after fear conditioning. In this regard, it was also assumed that the visceral CS-US association would be acquired more rapidly compared to the somatic modality. We also presumed (Hyp.2b) that the extinction of visceral pain-related fear responses would be more fragile, i.e., less efficient and more susceptible to interference by selective reinstatement, compared to extinction of somatic pain-related fear responses.

The first important finding of this study, was that modality-specific pain characteristics distinctively shape associative learning of behavioral and neural pain-related fear responses during both acquisition and extinction. In detail, CS predicting visceral pain were associated more rapidly and resulted in more negative CS valence ratings

compared to the CS^- as well as compared to the somatic pain-predictive CS^+, in line with the first hypothesis (Hyp.2a). However, awareness of CS-US contingencies was accurate in all learning phases, suggesting a relevance of modality-specific pain aspects in conditioned emotional responses, but not in cognitive aspects of associative learning.

This result is well in line with the concept of preparedness, proposing a more rapid acquisition resulting in enhanced emotional responses for stimuli with higher biological significance compared to less salient stimuli (Ohman et al., 2001). As reflected in the enhanced perceived unpleasantness of visceral US compared to somatic US, the visceral US is highly likely to display the stimulus with higher salience herein. Similar results have been reported in the context of selective learning of CS based on their salience in animal (Holland, 1999) and human studies (O'Tuathaigh et al., 2003), referred to as the "overshadowing effect" (Mackintosh, 1976). The "overshadowing effect" explains selective learning of more salient CS based on their relevance to survival (Mackintosh, 1976) as an adaptive strategy in order to react quickly to the stimulus with the highest threatening value in the face of multiple competing stimuli and limited processing capacity (Holland, 1999). Salience, in this regard, was not directly attributable to differential perception of pain intensity or pain unpleasantness of the US as revealed by covariation analyses, but may rather reflect the biological significance based on the associated risk for survival of the visceral pain stimulus used as a US here. Hence, novel evidence is provided here that the preparedness theory is not only referable to the biological significance of CS but can also be applied to differential salience and/or aversiveness of distinct painful US in differential fear conditioning in humans.

Interestingly, this overshadowing-like effect driven by US salience did not only lead to higher conditioned responses for the more salient visceral CS, but to a complete absence of emotional learning for the less salient somatic CS-US association, despite correctly assigning the somatic pain-predictive CS to the somatic US. Indeed, the CS^+_{SOM}-induced behavioral and neural responses were virtually identical to the non-pain-predictive CS^-, supporting that the pain-predictive CS^+_{SOM} – despite its predictive value – turned into a second "safety" cue. This was an unexpected finding given the somatic pain stimulus being perceived as unarguably painful and hence, aversive, which was expected to induce significant anticipatory conditioned emotional responses. Impressively, the presence of the more salient visceral CS-US association seemingly

leads not only to an overshadowing-like effect but presumably to a full suppression of learned emotional responses for the less salient somatic CS-US pair, suggesting a devaluation of the aversiveness of the somatic US compared to the significantly more unpleasant visceral US.

On a neural level, analyses revealed differential engagement of regions associated with pain anticipation or pain processing (S1/M1; Iannetti et al., 2010) as well as in insular and cingulate regions as part of a salience network (Menon et al., 2010; Wiech et al., 2010) during acquisition. These regions are consistent with other data showing enhanced neural activation for CS^+ compared to CS^- in differential fear conditioning (Büchel et al., 2000; Fullana et al., 2018; Sehlmeyer et al., 2009). Of note, differential responses in these brain areas were mainly driven by deactivation to the CS^+_{VIS}, which has seldom been reported in fear conditioning studies (Büchel et al., 2000; Fullana et al., 2018; Sehlmeyer et al., 2009) and is critically discussed later (see 5.4.).

In this study, enhanced CS^+_{VIS}-induced neural responses were not only observed compared to the CS^-, but also compared to the somatic pain-predictive CS^+, substantiating a role of pain modality in acquisition of conditioned anticipatory emotional responses. The findings, however, seems at odds with earlier fear conditioning studies (Sehlmeyer et al., 2009) reporting that distinct neural responses in the insula and ACC are independent of different CS/US characteristics. Notably, as pointed out before, these reviews only included a small number of experimental models using visceral pain and most importantly did not compare learning across pain modalities. In line with the notion that the somatic CS^+ has likely gained the emotional attributes of a second safety cue, similar to the CS^-, differential involvement of cingulate and insular regions could also display enhanced preparatory processing of cues predicting a stimulus of high salience (visceral pain) compared to a cue predicting any less salient event (somatic pain or no pain).

Interestingly, additional differential neural activation was observed in the thalamus, but only for the CS^+_{VIS} compared to the CS^-, suggesting that the thalamus may be specifically involved in differentiating between pain-predictive and non-pain-predictive cues, but not between pain modalities. Furthermore, there was no differential neural activation in the amygdala. This is in accordance with some newer reviews of human

fear conditioning (Fullana et al., 2018; Sehlmeyer et al., 2009), but at odds with older reviews (Büchel et al., 2000). Notably, amygdala activation is reported only in some studies within the somatic (Fullana et al., 2018; Sehlmeyer et al., 2009) and even less often within the visceral brain imaging literature ((Icenhour et al., 2015), but see also (Kattoor et al., 2013)). Recently, involvement of the amygdala in fear acquisition has been discussed to not reflect a subjective feeling of fear, but rather to the perception of threat, which is suggested to be rather shaped by the arousal induced by the pain stimulus (for more information, see LeDoux, 2014). Hence, in this study, the absence of a differential effect in the amygdala might also be due to comparable levels of arousal across modalities.

In line with the second hypothesis (Hyp.2b) that the extinction of visceral pain-related fear is more susceptible to interference by selective visceral reinstatement, valence ratings of the pain-predictive CS^{+}_{VIS} remained more negative compared to CS^{-} after unexpected visceral pain stimulation following extinction. Moreover, in the visceral reinstatement group enhanced neural deactivation for the CS^{+}_{VIS} compared to the CS^{+}_{SOM} and CS^{-} was observed in regions associated with salience processing (anterior insula, ACC; Menon et al., 2010; Wiech et al., 2010), in the "extinction network" (amygdala, hippocampus; Fullana et al., 2018; Hermans et al., 2006), as well as in areas previously observed to be associated with modality-specific aspects of pain anticipation (posterior insula). These brain regions have been reported in previous studies investigating reinstatement effects in differential fear conditioning paradigms using either only visceral US (Gramsch et al., 2014; Icenhour et al., 2015) or only somatic US (Lonsdorf et al., 2014), but have never been demonstrated to display modality-specific effects of pain-related fear reinstatement. Notably, no differences in CS-induced neural responses were detectable during the preceding extinction phase. Hence, the observed differences in neural activation likely reflect a return of conditioned differential responses for the visceral pain-predictive CS^{+}.

In the somatic reinstatement group, no differences in behavioral or neural CS-induced responses were detectable, albeit experimental studies have demonstrated successful reinstatement after presentation of even a few somatic pain stimuli (Lonsdorf et al., 2014). Complementing the idea that overshadowing rather displays a deficit in acquisition learning than in retrieval of fear memory (Holland, 1999), the failure of

reinstatement of conditioned responses to the somatic pain-predictive CS^+ in the somatic reinstatement group can most likely be explained by the absence of learned somatic pain-related fear responses during acquisition. Moreover, a reinstatement of visceral pain-predictive CS^+ was also not observed in the somatic reinstatement group. This finding implies that the suggested reactivation of conditioned responses is rather specific to the US associated with the particular memory trace during acquisition learning (Rescorla et al., 1972), and is not induced by just any unexpected painful experience.

In sum, these findings suggest that US modality (and presumably its salience) affects not only the acquisition but also the extinction efficacy, resulting in a return of conditioned differential responses on a neural level after inhibitory learning during extinction. However, behavioral findings were less unambiguous, rather suggesting a disruption of the extinction process. Hence, the reactivation of differential neural responses can presumably be associated with a return of fear responses for the visceral modality based on the present results, however with a few considerable limitations (see 5.4.). Interestingly, differential neural activation was observed in similar insular and cingulate areas during acquisition and after visceral reinstatement. These findings further imply that the role of pain modality in pain processing and differential pain-related fear learning is substantially mediated by key regions of the salience network, as discussed in the next section.

5.3. Role of the insula in modality-specific pain processing and pain-related fear learning

The findings of both studies suggest a crucial role and reciprocal connection between key regions of the salience network and adjacent insular areas in neural processing of modality-specific aspects in the context of pain and in pain-related fear learning. In the first study, a functional relation between the enhanced pain-induced activation in the anterior insula and in the dACC and anticipatory deactivation in the posterior insula was observed for the visceral compared to the somatic modality. In the second study, the intricate role of the insula in processing modality-specific aspects was further explored, suggesting that posterior insula deactivation to the visceral pain-predictive CS^+ is presumably a result of an associative learning process, reflecting adaptive, preparatory

responses in the anticipation of visceral compared to somatic pain. Interestingly, this view was further substantiated as differential posterior insula deactivation was selectively reactivated by visceral, but not by somatic reinstatement.

These findings imply potentially distinct roles of posterior and anterior parts of the insula in anticipation and processing of modality-specific pain aspects, potentially with a functional anterior-to-posterior shift in the process of learning to differentiate pain-predictive cues signaling visceral pain from somatic pain-predictive cues. This process may be driven by the enhanced salience of visceral pain stimuli in order to quickly and adequately react to cues signaling the more salient stimulus based on their biological significance, in line with the preparedness concept (Ohman et al., 2001). In this process, key regions of the salience network, i.e., the anterior insula and the dACC, might play a role in detection and evaluation of the more salient stimuli (Iannetti et al., 2010; Mayer et al., 2015; Menon et al., 2010; Wiech et al., 2010), herein visceral pain.

Usually, as reported in the pain literature, the posterior insula is rather associated with sensory-discriminative aspects of pain processing than with cognitive and emotional learned responses in anticipation of pain (Ploghaus et al., 1999). Our findings suggest that posterior parts of the insula may additionally be involved in processing of modality-specific differences between painful stimuli, likely based on their salience. Moreover, engagement of the posterior insula has also been discussed frequently in terms of regulation and recovery of the body's homeostasis (Craig, 2009). One may speculate that after pain-related fear learning has been accomplished, anticipatory insular activation reflects a representation of homeostasis regulation (Menon et al., 2010) in a posterior-to-anterior fashion, i.e., that salient pain-predictive cues are identified and processed in more posterior nodes and information is then transferred to and integrated in more anterior nodes in the bilateral insulae (Craig, 2011). Consecutively, information might be further processed in the ACC with (a) regard to evaluation of emotional and cognitive pain-specific characteristics, such as the saliency of the stimulus and (b) preparation and execution of responsive cognitive strategies, such as coping (Menon et al., 2010). However, the specificity of this functional connection for pain processing is still under current debate (Lieberman et al., 2015) and the salience network is most likely also involved in other tasks requiring stimulus selection based on salience. Interestingly, adaptive, preparatory responses in insular and cingulate areas can be

selectively reactivated after brief exposure to the salient, visceral pain stimulus, presumably in order to reinstall the necessary regulatory processes in the anticipation of visceral pain.

Of course, the exact interaction between posterior and anterior insula nodes and the dACC from the anticipation to processing of pain cannot be conclusively defined based on the presented data. However, the presented findings suggest a potential role of the posterior insula and key nodes of the salience network in differentiation of modality-specific pain characteristics and pain-related fear learning processes. Since activation of insular and cingulate regions has been reported to be altered in chronic pain patients in response to pain (Brown et al., 2014; Lloyd et al., 2016; Mayer et al., 2015) and during learned pain anticipation (Icenhour et al., 2015; Labus et al., 2013; Brown et al., 2014; Loyd et al., 2016), the reported findings on the role of pain modality have important implications for a better understanding of pain mechanisms and fear memory circuitries assumed to be involved in chronic pain.

5.4. Limitations

Of note, the presented findings strongly imply that behavioral and neural responses obtained using somatic pain cannot be unrestrictedly generalized to perception and processing of visceral pain. However, the reported differences in behavioral and neural responses may be restricted to the pain application methods used and the body regions stimulated in both studies, i.e. to rectal distensions (interoceptive, visceral pain) compared to thermal heat stimuli applied to the volar forearm (exteroceptive, somatic pain). Therefore, it cannot be excluded that the differences in invasiveness/aversiveness of these experimental pain models may have contributed to, for instance, the higher unpleasantness of interoceptive, visceral pain observed in both studies. Other research methods that may seem more refined yet are also more invasive, exist, comparing interoceptive visceral and interoceptive somatic pain induced by distensions of the distal (visceral) compared to the proximal (somatic) part of the esophagus, respectively (Aziz et al., 2000) or in closer proximity of body regions (Strigo et al., 2003). The findings of these few studies, however, are in line with our results of differential behavioral (Dunckley et al., 2005) and neural responses to visceral relative to somatic pain (Aziz et al., 2000; Dunckley et al., 2005; Strigo et al., 2003), suggesting that modality-specific

aspects such as emotional-affective pain aspects or salience shape pain processing independent of stimulus application or methods used.

Of note, studies in the field of somatic pain research have even suggested that different regions assigned to somatic body areas might also be distinct in terms of salience based on their supposedly differential relevance for survival, for instance, when they are closer to essential organ systems, compared to others. Especially the trunk or face region might be perceived differently compared to the upper and lower limbs with regard to sensory-discriminative pain aspects (K Schmidt et al., 2016; K. Schmidt et al., 2015). In the light of these findings and in accordance with the early proposal of Cervero (1999), the distinction of the entire psychophysiological system of the human body into only two different pain modalities seems, albeit heuristically necessary, overly simplified. Instead, it seems more likely that the pain experience is most probably not limited to a binary pain modality system, but shaped by the respective body region based on its underlying psychophysiological mechanisms. Therefore, further research is warranted using different experimental pain models to further investigate the role of pain modality for different body areas. Given that salience seems to be a relevant factor driving differences in pain processing and pain-related learning, more research is needed aimed at examining the role of salience between as well as within pain modalities.

The pain experience is multidimensional and complex and it is acknowledged that there are several interacting physiological and psychological factors affecting pain perception and processing (Gatchel et al., 2007). Therefore, other psychological aspects, such as catastrophizing or stress (Elsenbruch, 2011), which were not investigated herein, could also potentially contribute to the enhanced emotional responses to visceral compared to somatic pain. Especially the impact of psychological and physiological stress, which is known to mediate visceral pain-related responses (Elsenbruch, 2011) seems interesting in this context with regard to a potential contribution of HPA-axis activation to enhanced emotional conditioned responses in the anticipation of visceral compared to somatic pain.

Furthermore, as demonstrated in the two studies, enhanced neural deactivation was observed during the cued anticipation of visceral pain, which was replicable for the posterior insula across different data sets. The deactivation of the BOLD signal reported

in this study is based on estimated negative percent signal changes extracted based on well-established analysis methods (Glascher, 2009). In the context of pain, deactivations are seldom reported, albeit a critical review has suggested deactivations to pain stimulation might be induced in a number of brain regions often associated with pain processing (Kong et al., 2010). However, the exact mechanisms behind BOLD deactivations remains insufficiently understood and can only be carefully interpreted as neural deactivations resulting from decreased cerebral blood flow (Kong et al., 2010). Moreover, missing reports of deactivation in fMRI studies can potentially be explained by a lack of reporting percent signal changes, which can clarify the factors driving the observed differential effects in neural responses, presumably affecting the interpretation of often reported differential neural responses observed during pain stimulation or pain-related fear learning.

Moreover, the interpretation of the reactivated behavioral and neural responses is limited by the fact that no consolidation windows were implemented between acquisition, extinction and reinstatement phases. The inclusion of time windows between learning phases is discussed to be relevant for the establishment of long-term potentiation processes (LTP) to transfer short-term into long-term memories. During these time windows, alterations in neurobiological mechanisms have been demonstrated on a cellular level in relevant areas associated with fear conditioning, such as the amygdala (Johansen et al., 2011). As certain time windows are necessary (mostly set at 24 hours between learning phases) to consolidate fear memories (Johansen et al., 2011), it seems arguable that factually no reactivation of conditioned pain-related fear responses was observed in the second study, but rather a disruption of the extinction process by unexpected pain application for the visceral modality. This is partly confirmed by the enhanced negative valence of visceral CS^+ even after the extinction phase, albeit no differences in neural activation were detectable between pain-predictive CS^+ during extinction. Although this aspect needs further clarification, the disruption of extinction displays a relevant process in the clinical reality of chronic pain patients, as explained further within the next section.

5.5. Clinical implications and future directions

The reported findings suggest that pain modality shapes the experience of acute pain in healthy adults. Naturally, the investigation of pain-related fear learning and memory mechanisms in healthy participants is necessary to understand the behavioral and neural basis of the associative learning processes relevant for the acquisition and maintenance of chronic pain. Moreover, recent evidence suggests that neural responses in brain areas associated with memory processing and extinction learning are altered in patients with chronic pain syndromes (Icenhour et al., 2015; Labus et al., 2013; Lloyd et al., 2016), suggesting pathophysiologic processes mediating neural pain-related fear responses. Given that different chronic pain conditions are characterized by distinct pain modalities, pain-related symptoms specific for the given condition are likely also shaped by modality-specific pain aspects. Specifically, emotional-affective pain characteristics, such as the great discomfort accompanying visceral painful sensations, could be more prominent in visceral chronic pain conditions, such as in irritable bowel syndrome. In chronic pain syndromes primarily shaped by somatic pain, exaggerated perception of sensory-discriminative pain aspects, such as intensity may be at least equally relevant to the symptom complex.

The increased sensitivity or responsivity to pain, i.e., hyperalgesia, is a characteristic symptom in the context of chronic pain (Gebhart, 2000). Sensitization processes with regard to increasing discomfort or agony, however, have been studied less extensively. Given the multitude of factors contributing to visceral hyperalgesia along the brain-gut-axis (Elsenbruch, 2011), future studied are warranted to investigate potential differences in psychophysiological mechanisms underlying visceral compared to somatic hyperalgesia (as suggested by Cervero, 2009 or Gebhart et al., 2016), especially with respect to emotional-affective pain aspects. Moreover, recent evidence has suggested differences in mid- and long term sensitization processes on spinal and supraspinal levels, such as “wind-up” and LTP for distinct pain modalities (Cervero et al., 1999). Therefore, the specificity of visceral pain should be further elucidated with regard to endogenous modulatory pain mechanisms (Sandkuhler et al., 2012) suggested to be underlying hyperalgesia that are known to be relevant for the pathophysiology of chronic abdominal pain syndromes, such as IBS.

As demonstrated in study 2, the unique characteristics of visceral pain mediate the acquisition of conditioned pain-related fear responses on a behavioral and neural level. Albeit based on findings in healthy women, these findings suggest specific attentional and emotional resources involved in associative learning processes relevant for the acquisition and maintenance of visceral chronic pain as proposed by the fear avoidance model (Vlaeyen, 2015). Furthermore, the perpetuating vicious cycle driven by negative emotions and cognitive distortions described this translational model, might be different for the transition from acute to chronic pain shaped by distinct pain modalities. Specifically, emotional aspects of associative learning and exaggerated pain perception might play a specific role in the patho-psychophysiological processes underlying chronic abdominal pain. This also has important implications for translational pain research, calling for more relevant experimental pain models, using carefully selected pain stimuli as close as possible to the clinical reality of chronic pain conditions. From the same perspective, the use of two (or more) competing US in a differential fear conditioning paradigm (along with the use of more complex visual settings for CS, (Hermann et al., 2016)), can be advantageous in order to examine fear learning and memory processes closer to the much more complex and conflicting settings in real-life situations.

Interestingly, modality-specific pain characteristics were demonstrated not only to affect acquisition learning, but also the return of conditioned emotional responses to visceral pain-predictive cues after selective reinstatement. From a clinical perspective, unexpected pain episodes are thought to promote the return of conditioned emotional responses, such as pain-related fear, and re-establish pain-related fear memories, further increasing the chance of re-occurring maladaptive cognitive processes and disabling pain-related behavior (Haaker et al., 2014). In this context, painful episodes can occur frequently and unexpectedly after curative treatment, but also during the time of therapy, potentially disrupting the therapeutic process. The reported findings imply that similar to the return of fear responses (Icenhour et al., 2015; Lonsdorf et al., 2014), inhibitory learning during the extinction of pain-related fear can also be disrupted by just a few painful episodes. Albeit this seems to further limit the effectiveness of therapy based on extinction learning, the disruptive effect is presumably specific to the body region of the associated pain stimulus and not generalized across modalities. In

order to improve the long-term effectiveness of cognitive-behavioral therapy in chronic pain, a better understanding of factors mediating or disrupting extinction learning is required calling for more studies investigating reinstatement effects in healthy and clinical populations.

As outlined, the insula and its functional subregions together with other key nodes of the salience network seem to be engaged in a number of psychological processes, some of which may be highly relevant in the context of pain and fear memory. As demonstrated in the two consecutive studies, the posterior insula is strongly suggested to be involved in modality-specific aspects of learned anticipatory fear responses, most likely reflecting aspects of distinct salience across modalities. Based on previous findings linking altered pain anticipation to modified pain processing in patients with chronic pain conditions (Brown et al., 2014; Lloyd et al., 2016; Mayer et al., 2015), a better understanding of this functional connection may be of high relevance for a better understanding of chronic pain mechanisms. Therefore future research is warranted to specify the putative role of the insula in modality-specific aspects of pain-related fear learning processes associated with the acquisition and maintenance of chronic pain syndromes.

Based on the behavioral and neural findings from this thesis, the complex and multidimensional experience of pain seems to be mediated by unique psychophysiological aspects of the visceral compared to the somatic pain modality in healthy adults. Specifically, interoceptive, visceral pain is strongly suggested to be of higher salience compared to exteroceptive, somatic pain, which evidently shapes neural mechanisms underlying learning, and extinction, and potentially also the reactivation of pain-related fear. However, the relevance of modality-specific effects to pathophysiologic mechanisms in patients with different chronic pain syndromes, especially with regard to extinction and reactivation of pain-related fear, remains incompletely understood. Therefore, different and clinically-relevant experimental pain models should be utilized to further elucidate the role of pain modality in pain-related fear learning and memory mechanisms relevant for the transition from acute to chronic pain and improve cognitive-behavioral therapy of chronic pain conditions shaped by distinct pain modalities.

Summary

Learning to identify and memorize events that predict pain serves as an adaptive strategy to avoid painful episodes, restore homeostatic function and sustain health. In the context of chronic pain, however, maladaptive associative learning processes can promote negative emotional responses in the anticipation of pain, such as pain-related fear. Different chronic pain syndromes are shaped by pain arising from distinct body regions, which can be assigned to either the interoceptive, visceral or exteroceptive, somatic pain modality. The different psychophysiological principles underlying distinct pain modalities are suggested to shape differential pain perception and processing. However, the role of pain modality, especially in pain-related fear learning and extinction, has not been systematically examined yet.

Two consecutive functional magnetic resonance imaging studies were conducted. In the first study, well-established experimental pain models were used to compare visceral and somatic pain stimuli, which were individually-matched for perceived pain intensity. This study revealed shared brain activation across modalities, but also enhanced neural responses to visceral compared to somatic pain in areas associated with emotional-affective and salience pain aspects, suggesting a higher biological significance of visceral pain. In the second study, a differential fear conditioning paradigm was implemented using visceral and somatic pain as competing unconditioned stimuli (US), signaled by two distinct pain-predictive conditioned stimuli (CS^+), respectively, compared to a non-pain-predictive stimulus (CS^-). Herein, a more rapid acquisition and enhanced pain-related fear responses to visceral pain-predictive CS^+ were demonstrated as well as a return of fear only for the visceral modality. Notably, both studies suggested a relevance of cingulate and insular cortices, specifically of the posterior insula, in modality-specific aspects of pain-related fear learning.

Together, both studies strongly suggest a role of pain modality in pain processing and pain-related fear learning and memory mechanisms and underline the importance of clinically-relevant experimental pain models in translational pain research. Future research in chronic pain patients is warranted to further elucidate the pathophysiology underlying chronic pain syndromes shaped by distinct pain modalities.

Zusammenfassung

Situationen oder Reize identifizieren zu können, nach denen potentiell Schmerzen auftreten, stellt einen adaptiven Lernprozess dar, um weitere Schmerzen zu vermeiden, das homöostatische Gleichgewicht aufrecht zu erhalten und die körperliche Unversehrtheit zu gewährleisten. Im Zusammenhang mit chronischen Schmerzen können maladaptive Lernprozesse jedoch negative Emotionen, wie schmerzassoziierte Furcht, schon während der Schmerzantizipation begünstigen. Unterschiedliche chronische Schmerzsyndrome werden durch Schmerzen aus verschiedenen Körperregionen geprägt, die der interozeptiven, viszeralen oder exterozeptiven, somatischen Schmerzmodalität zuzuordnen sind. Diesen Schmerzmodalitäten liegen spezifische, psychophysiologische Prinzipien zu Grunde, die sich vermutlich differenziell auf die Schmerz-wahrnehmung und -verarbeitung auswirken. Der Einfluss der Schmerzmodalität auf Lernen und Extinktion von schmerzassoziierter Furcht wurde bislang jedoch nicht systematisch untersucht.

Zu diesem Zweck wurden zwei aufeinanderfolgende funktionelle Magnetresonanztomographie-Studien durchgeführt. In der ersten Studie wurden mithilfe etablierter experimenteller Schmerzmodelle die Wahrnehmung und Verarbeitung von viszeralen und somatischen Schmerzreizen verglichen, die zuvor hinsichtlich der Schmerzintensität individuell angepasst wurden. Hierbei ließ sich eine vergleichbare Aktivität für beide Modalitäten aufzeigen, insbesondere aber eine erhöhte schmerzinduzierte Aktivität für die viszerale Modalität in Hirnarealen, die mit der Verarbeitung von Emotionen/Affekt und Salienz im Kontext akuter Schmerzen assoziiert werden. Diese Ergebnisse sprechen für eine höhere biologische Salienz viszeraler im Vergleich zu somatischen Schmerzen. In der zweiten Studie wurde ein differentielles Furchtkonditionierungs-Paradigma implementiert, in dem viszerale und somatische Schmerzreize als unkonditionierte Stimuli (US) jeweils von unterschiedlichen schmerzprädiktiven Warnsignalen (CS^+) angekündigt wurden, verglichen mit einem nicht-schmerzprädiktiven Stimulus (CS^-). Hier zeigte sich ein schnellerer Erwerb stärker ausgeprägter Furchtreaktionen auf viszerale schmerzprädiktive CS^+, sowie eine Reaktivierung von Furchtreaktionen ausschließlich für die viszerale Modalität. Die Ergebnisse beider Studien weisen zudem auf eine besondere Relevanz des cingulären und, insbesondere posterioren, insulären Cortex, für modalitätsspezifische Aspekte des Lernens schmerzassoziierter Furcht hin.

Insgesamt demonstrieren beide Studien die zentrale Rolle der Schmerzmodalität in der Schmerzverarbeitung sowie bei Lern- und Gedächtnisprozessen im Kontext schmerzassoziierter Furcht. Die Ergebnisse heben außerdem den Stellenwert klinisch bedeutsamer, experimenteller Schmerzmodelle für die translationale Schmerzforschung hervor. Dennoch bedarf es weiterer Studien, insbesondere mit Patienten mit chronischen Schmerzen, um die Pathophysiologie unterschiedlicher Schmerzsyndrome, die durch verschiedene Schmerzmodalitäten geprägt werden, besser zu verstehen.

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Figures

Tables

List of abbreviations

ACC	anterior cingulate cortex
ACQ	acquisition
Base	baseline
BMI	body-mass-index
BOLD	blood-oxygen-level-dependent
°C	degree Celsius
CS	conditioned stimulus
cm	centimeters
CNS	central nervous system
CR	conditioned reaction
dACC	dorsal anterior cingulate cortex
dlPFC	dorsolateral prefrontal cortex
dmPFC	dorsomedial prefrontal cortex
EDA	electrodermal activity
ENS	enteric nervous system
EXT	extinction
Fig.	figure
FOV	field of view
FWE	family-wise error
FWHM	Full Width at Half Maximum
GI-tract	gastrointestinal tract
GRAPPA	generalized auto-calibrating partially parallel acquisitions
HPA-axis	hypothalamic-pituitary-adrenal axis
hrf	hemodynamic response function
IBS	irritable bowel syndrome
LCC	locus coeruleus
LTP	long term potentiation
M1	primary motor cortex
MCC	midcingulate cortex
ME-EPI	multi-echo echo planar imaging
mm	millimeters
mmHg	millimeter of mercury
MNI	Montreal Neurological Institute
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MRI	magnetic resonance imaging
NS	neutral stimulus
PAG	periaqueductal gray
PCC	posterior cingulate cortex
PFC	prefrontal cortex
pgACC	perigenual anterior cingulate cortex
PPC	posterior parietal cortex
RE	reinstatement
rmANOVA	repeated measures analysis of variance
ROI	regions of interest
s	seconds
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
SEM	standard error of the mean
SOM	somatic
SPSS	Statistical Package for the Social Sciences
Tab.	table
TE	echo time
TR	repetition time
UR	unconditioned reaction
US	unconditioned stimulus
VAS	visual analogue scale
VIS	visceral
vlPFC	ventrolateral prefrontal cortex
vmPFC	ventromedial prefrontal cortex
WDR	wide dynamic range

Acknowledgements

First and foremost I would like to express my immense gratitude to Prof. Dr. Sigrid-Elsenbruch-Harnish, my research supervisor, for her competent guidance, her always insightful and constructive criticism, and her encouragement, when I needed it most. It is thanks to her outstanding support that I have been able to grow both scientifically and personally during this time. I am looking forward for the projects to come in the future.

I would also like to thank the other reviewers for taking the time to evaluate this thesis.

Also, I would like to thank Dr. Adriane Icenhour for her thoughtful advice, fruitful discussions and never-ending patience. She has always been a role model and has become my confidante, sharing my love for science and a good Primitivo.

Moreover, I would like to thank Prof. Dr. Sven Benson, Prof. Dr. Harald Engler, as well as my other dear colleagues. You have all motivated and supported me in one way or another, sometimes without even knowing, and it is a great pleasure to work with all of you.

I also want to say thank you to Prof. Dr. Jost Langhorst and Dr. Margarita Schöls as well as Prof. Dr. Ulrike Bingel, Dr. Katarina Forkmann and Dr. Nina Theysohn for their advice and support.

Special thanks to my partner in crime, Annika and to Sopiko for their support in data acquisition and for helping me keep my calm and also to Laura and Robert for cracking silly jokes and having an open ear on rainy days.

Furthermore, I want to express my deep and sincere thankfulness to my beloved family, the one I grew up with and the one I was blessed with over the years. For your kind words, your unconditional support, your wisdom and love. I could not have done this without you. Thanks to my lovely friends for sticking up with me, for freeing my mind at the Spa, on our “little Fridays” or over a cold “Kölsch and Metthappen”.

At last and most importantly I would like to thank you, Frank, for simply everything. Words cannot express how endlessly grateful I am that you supported me all this time, that you have comforted me at my worst and pushed me at my best. I have not regretted one day I sat in the train back home to you.

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