Discrimination, conditioning and generalisation: Testing the assumptions of the imprecision hypothesis of pain

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Summary

Pain is usually a response to actual or potential threat. However, for some people, pain persists long after the actual or potential threat has resolved. Although the understanding of pain has rapidly advanced over the last two decades, scientists and clinicians are still unable to fully explain why pain persists. Most research in pain has focused on the development of sensitivity in the “neural pathways” that transmit news of potentially dangerous events, yet this sensitivity does not explain many persistent pain states.

A recent approach to the persistent pain problem is the imprecision hypothesis of chronic pain. The imprecision hypothesis of chronic pain proposes that associations that form between nociceptive signals and non-nociceptive signals, through associative learning, might contribute to the development of persistent pain. That is, through classical conditioning—a form of associative learning made famous by Ivan Pavlov—a nociceptive signal could become associated with other accompanying sensory inputs or contextual cues. As such, the sensory input or contextual cues become capable of eliciting a pain response without nociception.

This thesis investigates some of the underlying theoretical assumptions of the imprecision hypothesis of chronic pain. Chapter 1 discusses the theoretical assumptions of the imprecision hypothesis of chronic pain and focuses in on the assumptions addressed in this thesis. Chapter 2 outlines the preparatory work and piloting carried out to ensure the experimental procedures were robust. Chapter 3 investigates the evidence for whether cognitive manipulations can be used to selectively modulate the sensory and affective dimensions of pain. Chapter 4 investigates the researchers' assumption that participants will order a sequence of visual stimuli the same way, and with the same result, as researchers do. Chapter 5 investigates the extent to which conditioning-induced hyperalgesia generalises to similar but novel stimuli in healthy people. Chapter 6 investigates whether participants can actually distinguish between similar stimuli using a previously-published paradigm that employs pictures of faces. Chapter 7 investigates the extent to which conditioning-induced hyperalgesia generalises to similar but novel face stimuli in healthy people. Chapter 8 discusses how the studies in this thesis inform the imprecision hypothesis of chronic pain, and the wider implications for both future studies and clinical practice.

Declaration

This thesis presents work carried out by myself and does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; to the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, are clearly acknowledged.



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Preface

This thesis contains eight chapters. Each chapter is written so that it can be read independently. There are a few points to note regarding this thesis: (1) it includes traditional thesis chapters, three submitted manuscripts, which have been judged by the supervisory team as 'submission ready', and two manuscripts to be submitted. (2) The pages of the manuscripts are not consistent with the thesis pages as outlined in the Table of Contents. That is, the thesis page numbers stop where a manuscript is embedded and begin again after the manuscript. (3) Appendices and supplementary material for each manuscript appear directly after the manuscript, not at the end of the thesis. However, the protocols, as outlined in the Table of Contents, appear at the end of the thesis. (4) The reference list in Chapter 9 contains only the in-text references for the traditional thesis chapters, not the manuscripts. Each manuscript has its own reference list at the end. (5) Chapter 2 outlines the piloting process for the experiments contain in this thesis. At the beginning of each chapter the results from the piloting are presented.

1 Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

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Publications, presentations and awards

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Appendices

1. Introduction

Persistent pain affects an estimated 35.5% of the global population (Gaskin & Richard, 2012; Vos et al., 2013). In the Australian context, persistent pain affects one in five Australians and exerts a financial burden on society that is greater than that of diabetes and cancer combined (Blyth et al., 2001). Despite these alarming figures, the effectiveness of treatment of persistent pain is poor, with even the best treatments only moderately reducing pain when compared to control treatments (Henschke et al., 2010; Turk, 2002; Turk et al., 2003). Particularly concerning is that a high percentage of people (60%) are still in pain 12 months after its initial onset (Costa et al., 2009).

Traditionally, pain was viewed as a hierarchical system of sensory input, processing and motor output (Melzack & Wall, 1967). However, this hierarchical system cannot explain conditions such as phantom limb pain, complex regional pain syndrome and fibromyalgia. The field of pain research was shifted towards a less reductionistic view of pain by Melzack and Wall's landmark papers on gate control theory (Melzack & Wall, 1962, 1967). Now, fifty years later, it is widely accepted that pain is the result of an evaluative process whereby the brain weighs up a potentially massive mix of sensory inputs, and psychological and contextual cues, ultimately determining whether or not protective action will be beneficial (Moseley & Vlaeyen, 2015; Tabor, Thacker, Moseley, & Körding, 2017; Vlaeyen, 2015).

An early development in this reconceptualisation of pain was the acknowledgement of two key features of pain (Chapman & Gavrin, 1999). The first feature is that while nociception and pain are often highly correlated, they are, in fact, distinctly separate. That is, nociception is certainly a potent signal of threat, however, it is neither necessary nor sufficient for a pain experience to occur (Chapman & Gavrin, 1999; Wall & McMahon, 1986). The second feature is that pain has sensory and emotional characteristics. While pain is often expressed as a single, unitary experience, proposals emerged that suggested it has multiple dimensions (Price & Harkins, 1992a; Wade, Price, Hamer, Schwartz, & Hart, 1990), most commonly referred to as sensory and affective dimensions (Price & Harkins, 1992a).

The multidimensionality of pain is particularly relevant to the burden of pain on our communities (Price, 1999). That is, persistent pain disorders are characterised by the dominance of affective descriptions of pain. It seems that people with *unpleasant* pain are less likely to recover, and thus represent the bulk of those with persistent pain; or, pain becomes progressively more unpleasant as it persists (Price, Harkins, & Baker, 1987). This uncertainty points to an issue that forms a fundamental piece of the background of the current thesis: Why do some people recover from an acute episode of pain when others do not, and instead develop a chronic pain disorder?

While researchers have advanced their understanding of pain, the reasons why some people recover and others do not remain elusive (Campbell et al., 2012). Explanations such as injury type, occupation and personality have been proposed as possible predictors (Chou & Shekelle, 2010). However, all these explanations are, at best, weak predictors of the development of persistent pain (Chou & Shekelle, 2010). Landmark research showing that peripheral and spinal cord circuitry undergo extensive reorganisation in the presence of persistent pain (Woolf & Salter, 2000) led to assumptions that this reorganisation was the cause of persistent pain. However, these suggestions regarding peripheral and spinal cord reorganisation do not adequately explain the majority of persistent pain presentations, for example, non-specific back pain or fibromyalgia (Moseley & Vlaeyen, 2015). Therefore, continued exploration of the reasons why acute pain may persist is warranted.

The current thesis investigates the assumptions and predictions of a recent proposal called the "imprecision hypothesis of chronic pain" (imprecision hypothesis; Moseley & Vlaeyen, 2015). In short, the imprecision hypothesis suggests that associative learning mechanisms might contribute to the development of chronic pain after an acute pain episode, by way of overgeneralisation of a conditioned pain response.

The rest of this chapter is organised as follows.

Section 1.1 introduces the fundamental assumptions and clinical observations underlying the imprecision hypothesis of chronic pain.

Section 1.2 discusses the role of associative learning in the imprecision hypothesis of chronic pain.

Section 1.3 discusses the evidence of modulation of pain by non-somatosensory factors.

Section 1.4 summarises the components of the imprecision hypothesis of chronic pain addressed in this thesis.

1.1 The fundamental assumptions and clinical observations underlying the imprecision hypothesis.

Two concepts form the basis of the imprecision hypothesis (Moseley & Vlaeyen, 2015). First, that pain is a response, not merely a stimulus, and second, that non-nociceptive and nociceptive stimuli are encoded together. The initial encoding of these stimuli forms a *cortical blueprint* for subsequent responses to similar events (Moseley & Vlaeyen, 2015). Imprecise encoding of a painful event results in an imprecise representation or an imprecise '*cortical blueprint'* of the multisensory stimuli (nociceptive and non-nociceptive) that encompass a painful event (Moseley & Vlaeyen, 2015). The less precise the representation of the multisensory stimuli, the greater the likelihood that similar events (and accompanying multisensory stimuli) will be capable of eliciting pain (Moseley & Vlaeyen, 2015).

1.1.1 Evidence of imprecise encoding in people with pain

Some clinical evidence has shown that people with persistent pain are imprecise in their perceptions (Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015). Clinicians and researchers have observed that people with persistent pain localise their pain imprecisely (widespread pain; Luomajoki & Moseley, 2011) and/or have an imprecise perception of the size of the painful body area (Bray & Moseley, 2011; Lotze & Moseley, 2007; Luomajoki & Moseley, 2011). These differences raise the question as to whether these observations are manifestations of imprecise encoding of a painful event. For example, a study (Moseley, 2008) has shown that people with persistent pain tend to perceive the painful body area to be larger than its actual size, and have more difficulties in distinguishing between movements, than healthy controls. This trend has also been observed in brain imaging studies, where persistent pain populations often show a re-organisation of both sensory and motor areas within the brain (Moseley & Flor, 2012). That is, in people with persistent pain, the representation of non-nociceptive stimuli within the sensory and motor areas of the brain appears to be disrupted (Bushnell, Čeko, & Low, 2013).

A pivotal study conducted by Harvie et al. (2015) showed that non-nociceptive cues influence the point of movement at which someone with chronic neck pain experiences their pain. This study used three virtual reality conditions to manipulate visual feedback to 24 participants with neck pain. The first used visual feedback to make it seem, to the participant, like they were turning their head more than they actually were (overstated). The second used visual feedback to make it seem like the participant was turning their head less than they actually were (understated), and the third used visual feedback that accurately reflected how much the participant was turning their head (baseline; Harvie et al., 2015). Participants were asked to turn their heads to the point of first onset of pain. The findings showed that, compared to baseline, participants reported pain earlier in the movement when the visual feedback *overstated* how much they turned their head, and later in the movement when the visual feedback *understated* how much they turned their head. This suggests that the visual cues given to participants influenced their experience of pain.

1.1.2 The role of sensory and affective dimensions of pain

As previously alluded to, pain is multidimensional (Price, 2000, 2002). The International Association for the Study of Pain (IASP) has identified that pain has sensory and affective dimensions (2002). The sensory dimension is described as the intensity, location and quality of pain (Price et al., 1987). The affective dimension is described as the unpleasantness of pain, sometimes described with emotional descriptors such as 'terrifying' (Price & Harkins, 1993). When persistent pain sufferers recall their pain experience, the affective qualities are more readily described than the specific sensory qualities (Edwards, Pearce, Collett, & Pugh, 1992). Consequently, the imprecision hypothesis considers not only the role of the sensory dimension of pain, but also the role of the affective dimension of pain (Moseley & Vlaeyen, 2015).

An assumption of the imprecision hypothesis is that a painful event that is dominated by the affective dimension may be more imprecisely encoded and subsequently imprecisely represented in the brain than a painful event that is not dominated by the affective dimension. This assumption is based on emotion literature on topics such as post-traumatic stress disorder (PTSD), in which proposed theories of development and reported symptoms of people with

PTSD resemble those of people with persistent pain (Runnals, Van Voorhees, & Calhoun, 2013). That is, PTSD is proposed to develop following an event that either is, or is perceived to be, threatening (Runnals et al., 2013). Similarly, pain is proposed to be a response to either actual or potential threat. People with PTSD often present with symptoms of responses to cues that resemble the stress event, such as avoidance, hyperarousal, increased startle response, difficulty with attention and hypervigilance (Runnals et al., 2013; Wald, Taylor, Chiri, & Sica, 2010). These presentations are also often observed in people with persistent pain (Gatchel & Epker, 1999). However, as with persistent pain, not everyone who experiences an extreme stress event develops PTSD (Otis, Keane, & Kerns, 2003). The post-traumatic stress disorder literature has proposed that heightened levels of affect, such as anxiety or panic, may increase the effect that the extreme stress event has on the person (Otis et al., 2003; Runnals et al., 2013; Yehuda, 2002). Moreover, the cognitive and information-processing model of PTSD outlines how heightened affect during the extreme stress event can disrupt the encoding of the event (Creamer, Burgess, & Pattison, 1992; Ehlers & Clark, 2000). This disrupted encoding can lead to the brain being unable to synthesise and accurately represent the initial extreme stress event (Ehlers & Clark, 2000). This can result in the stress response being elicited in the presence of cues that are similar to those which were present during the stress event (Ehlers & Clark, 2000). Therefore, the person with PTSD responds to both positive alarms of threat and false alarms of threat (Ehlers & Clark, 2000). Based on the similarities between PTSD and persistent pain, it is reasonable to assume that heightened affect may play a role in how precise the encoding of a painful event is (Moseley & Vlaeyen, 2015).

That sensory and affective dimensions are probably differentially involved in various pain episodes is consistent with the idea that these dimensions can be differentially modulated (Price, 1999). However, a common perspective of pain is that it is a unitary experience. These seemingly contrasting perspectives raise a critical unsettled issue – are the two dimensions sufficiently distinct that they can be separated and differentially modulated? The evidence appears to be mixed. Some studies (e.g. Chapman et al., 2001) show that these two dimensions of pain are indistinguishable from each other, while other studies (Rainville, Carrier, Hofbauer, Bushnell, & Duncan, 1999; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Rainville, Feine, Bushnell, & Duncan, 1992) show that people are able to distinguish between these two

dimensions of pain. Furthermore, the studies that have shown that these dimensions *can* be distinguished have also shown that, when participants are engaged in distinguishing between the two dimensions, brain activity seems to vary in a predictable and biologically feasible way (Rainville et al., 1997). Therefore, while there is agreement that pain has both sensory and affective dimensions, this agreement does not extend to the degree to which they can be separated (Fernandez & Turk, 1992).

A prerequisite to determining whether heightened affect plays a role in how precisely a painful event is encoded and cortically represented, first we must determine whether pain is in fact separable, and whether it can be differentially modulated. Certainly, pharmacological studies have demonstrated that, after people with persistent pain are given analgesics, they report a decrease in the affective dimension of pain but not the sensory (Price, Von der Gruen, Miller, Rafii, & Price, 1985). A randomised-controlled trial (Kupers, Konings, Adriaensen, & Gybels, 1991) investigated whether participants with neurogenic and idiopathic pain rated the sensory and affective dimensions of pain differently before and after the administration of morphine. The results showed that, compared to the participants in the placebo group who received saline injections, the participants who received morphine injections showed a decrease in pain affect, but not in pain intensity. However, this result may be a demonstration of how analgesics modulate both primary and secondary affects (see Figure 1).

Primary affect is the 'moment-by-moment unpleasantness of pain' and can be closely correlated with pain intensity (Price, 1999, p. 49). That is, experiencing pain for a few minutes may be unpleasant because of the pain intensity (Price & Harkins, 1992b). However, pain of a *consistent intensity* may become more unpleasant if it continues for several hours (Wade et al., 1990). Secondary affect results from having pain for longer (Price, 2000). Therefore, in order to accurately investigate what occurs if affect was heightened at the time when a painful event was initially encoded, primary affect needs to be assessed. Additionally, use of analgesics during an acute event may hinder the encoding processes of nociceptive input. Therefore, in order to address the assumption of the imprecision hypothesis that heightened affect leads to imprecise encoding, this study investigates whether cognitive manipulations can allow for separation and

differential modulation of the sensory and affective dimensions of pain at the time of event encoding.

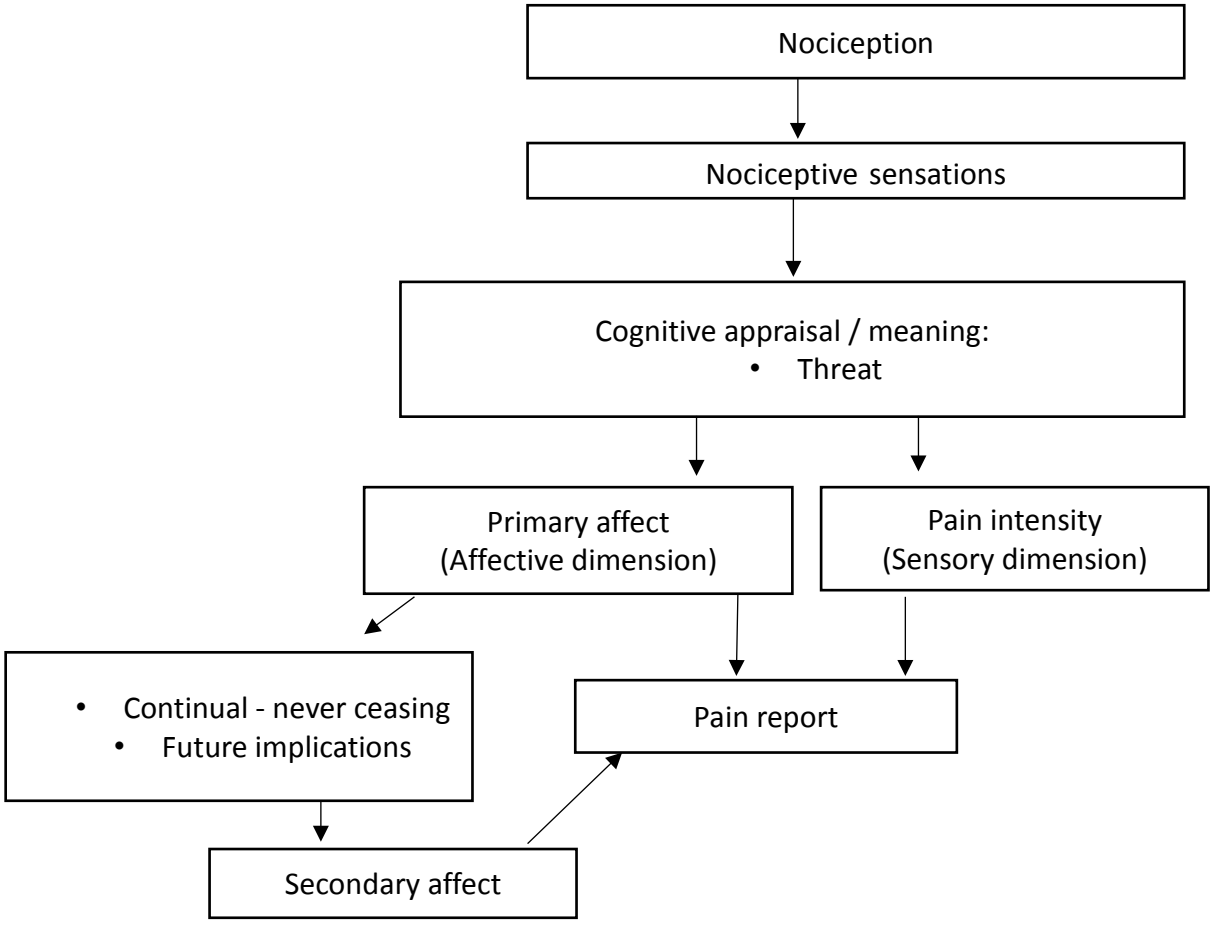


Figure 1. Graphical representation of nociceptive processing and the interaction between the different dimensions of pain, with particular emphasis on, the concepts of primary and secondary affect and their interaction, which contributes to pain expression. This image was adapted from Price (1999).

Related research question: *Can the sensory and affective dimensions of pain be selectively modulated using cognitive manipulation?*

The systematic review in Chapter 3, "Pain's sensory and affective qualities – differentially modifiable dimensions or two aspects of a unitary experience?", addresses this question.

1.1.3 Changing thesis direction to focus on generalisation

The thesis protocol outlined several experiments that would further investigate the role of affective state in encoding. However, when the above research question was answered, it became clear that a substantial amount of work would be required to find a method of experimentally manipulating the affective dimension in order to investigate encoding. Therefore, this thesis took a different direction and focussed instead on the role of stimulus generalisation and perceptual discrimination in the imprecision hypothesis.

1.1.4 Generalisation

Generalisation is often defined differently by different authors. Hull (1943) defined generalisation as "…the reaction involved in the original conditioning connected with a considerable zone of stimuli other than, but adjacent to the stimulus involved in the original conditioning" (Hull, 1943, p. 183). Hilgard and Marquis (1961) defined it as "…the behavioural fact that a conditioned response formed to one stimulus may also be elicited by other stimuli which have not been used in the course of conditioning" (Hilgard & Marquis, 1961, p. 328). Finally, Dollard and Miller (1950) described it as "… once a response has been reinforced in one situation, the function of generalisation is to increase the probability that the response will occur in other similar situations" (Dollard & Miller, 1950, p. 1). While these definitions are seemingly similar, there is a difference in which aspect of generalisation is emphasised. The first emphasises the stimulus, the second emphasises the response and the third emphasises the process (Mostofsky, 1965). Nevertheless, regardless of how one defines generalisation or emphasises certain aspects of it, generalisation is an accepted feature of behaviour (Mostofsky, 1965).

The adaptability of responses such as fear and pain allows an organism to adjust, predict and avoid different threatening events (Pearce, 1987). The process by which this occurs is *generalisation*, and is thought to be ideal for survival because the organism can learn from danger and therefore avoid it in the future (Vlaeyen, 2015). Fear research has used a fear-conditioning paradigm to advance understanding of behaviours that are involved in conditions such as anxiety (Dunsmoor & Murphy, 2015; Lethem, Slade, Troup, & Bentley, 1983). This

research has shown that fear learning is never limited to the specific situation but that the learning is almost always extrapolated to similar situations, events or items (Davey, 1992). This results in the fear response being elicited in the presence of these situations, events or items that are similar to the original situation but essential novel (Davey, 1992).

Generalisation is key in anxiety disorders, particularly when the extrapolation of previous learning becomes excessive, such that the fear response is elicited by multiple classes of situations, events and objects (Haddad, Pritchett, Lissek, & Lau, 2012). This is often also seen in people with persistent pain, where all movements are reported as painful, so much so that people cease to use the painful body area (Costa et al., 2009). Therefore, generalisation is key to the imprecision hypothesis, which proposes that, if a painful event is imprecisely encoded, it will also be imprecisely represented in the brain, which will result in generalisation (Moseley & Vlaeyen, 2015; see Figure 2).

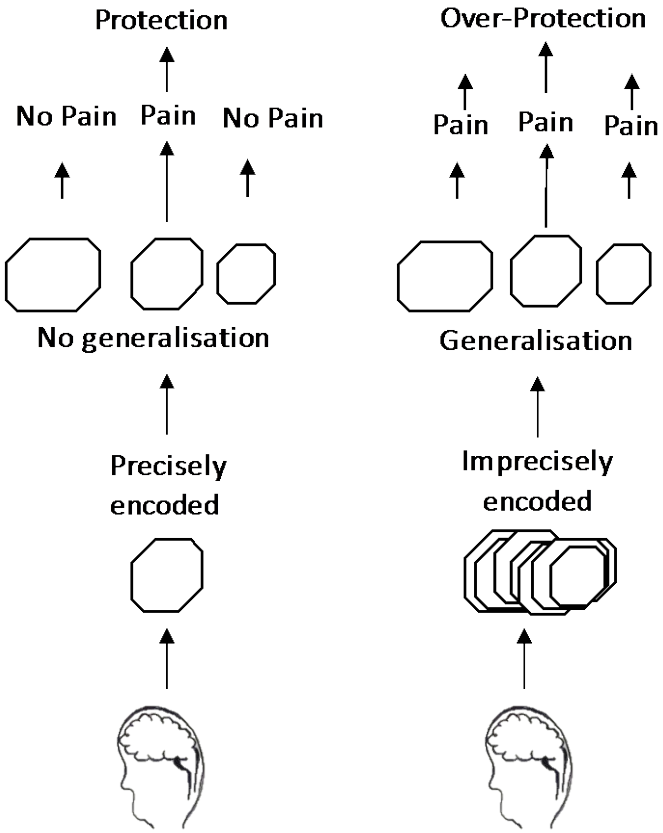


Figure 2. Graphical representation of the role of generalisation in pain (this image was adapted from Moseley and Vlaeyen, 2015). A painful event constitutes a compound stimulus with

multiple sensory inputs and contextual cues. When the painful event is precisely encoded, as depicted on the left side of the image, then only those specific sensory inputs and contextual cues will elicit pain. Therefore, pain remains adaptive and protective because it is only elicited by those specific sensory inputs and contextual cues that are true threats (Moseley & Vlaeyen, 2015). However, when the painful event is imprecisely encoded, as depicted on the right side of the image, then other similar sensory inputs and contextual cues can elicit pain. Therefore, pain becomes maladaptive and overprotective because it is elicited by multiple similar sensory inputs and contextual cues that are non-threats. The key feature in this hypothesis is that the ability to identify actual or potential threats or non-threats and respond appropriately is determined by the degree of precision with which the painful event was originally encoded and represented in the brain (Moseley & Vlaeyen, 2015).

1.1.4 Perceptual discrimination

Stimuli are composed of features on multiple dimensions (Goldstone, Landy, & Son, 2010). That is, a stimulus can be made up of more than one feature (e.g. white and bright) on more than one dimension (e.g. colour or brightness; Goldstone et al., 2010). The stimulus *package* comes to be recognised as a single event, even though it incorporates many features. For example, when one sees a red chair, this apparently single stimulus actually constitutes features in several dimensions – including its shape, size and colour. Similarly, when one bends over, this apparently single stimulus actually constitutes features in several sensory modalities – possible nociception and proprioception, vestibular and visual inputs, and non-sensory inputs that may be contextual or experiential.

The ability of an individual to distinguish stimuli that are different in at least one dimension is referred to as perceptual *discrimination acuity* (Goldstone, 1998; Goldstone et al., 2010; Zaman et al., 2015). As previously mentioned (Struyf, Zaman, Vervliet, & Van Diest, 2015; Vervliet & Van, 2015; Zaman, De Peuter, Van Diest, Van den Bergh, & Vlaeyen, 2016; Zaman et al., 2015) people with persistent pain seem to show poor discrimination acuity for a range of sensory inputs, including those in modalities that contribute critical features of a compound stimulus that evokes pain. Poor perceptual discrimination acuity will lead to imprecise encoding and

cortical representation of the event. In such cases, a wider range of compound stimuli may seem to resemble the initial stimulus, increasing the propensity to generalise (Moseley & Vlaeyen, 2015).

1.2 The role of associative learning in the imprecision hypothesis

1.2.1 Stimulus–response relationship in non-associative learning

The stimulus-response relationship has been shown to be malleable to both associative learning and non-associative learning (Bornhövd et al., 2002; Hommel, 1997; Salomons, Johnstone, Backonja, & Davidson, 2004). In non-associative learning, the response to a stimulus changes because of repeated presentation of the stimulus. For example, after repeated presentation of a stimulus, the response may decrease (habituation) or increase (sensitisation; Woolf, 2009). Sensitisation is a term used to describe the changes that occur in the nociceptive system. Peripheral sensitisation describes the process by which the response profile of primary nociceptors is enhanced (Woolf, 2009). Peripheral sensitisation occurs when inflammatory molecules, for example prostaglandin, activate specific receptors on the cell wall of nociceptors. This triggers an internal cascade of events that results in increased sensitivity of the nociceptor to mechanical and thermal stimuli (Moseley & Butler, 2017). This enhanced sensitivity manifests clinically as primary allodynia (non-noxious stimuli now trigger pain) and primary hyperalgesia (noxious stimuli cause more pain than normal; Moseley & Butler, 2017).

Central sensitisation occurs within the central nervous system. It describes the process whereby the response of spinal nociceptors to a stimulus is enhanced. Therefore, there is an increased response to nociceptive stimuli within the central nervous system. There is extensive evidence that central sensitisation involves non-associative learning at a synaptic and network level in the spinal cord (Moseley & Butler, 2017; Woolf, 2009), but the possibility that it also involves associative learning has not been ruled out. One interesting consideration is that basic animal studies show non-associative learning at a synaptic level, but these studies lack some ecological validity because they assume that nociceptive input occurs in isolation (Ben-Eliyahu et al., 1992). In vivo, nociceptive input is always accompanied by non-nociceptive input (Pearce & Bouton, 2001). Therefore, in vivo studies cannot exclude the possibility of associative learning processes contributing to central sensitisation (Moseley & Vlaeyen, 2015). This is important, because central sensitisation is widely held to be an important contributor to persistent pain, particularly following primary nerve trauma or dysfunction (Ji, Kohno, Moore, & Woolf, 2003). However,

while peripheral and central sensitisation are important processes, they do not fully explain persistent pain (Moseley & Vlaeyen, 2015).

1.2.2 Stimulus-response relationship in associative learning

In associative learning, an association forms between two or more stimuli to elicit a response (Vlaeyen, 2015). A paradigm used to study associative learning is classical or Pavlovian conditioning (Vlaeyen & Linton, 2012). The famous experiments conducted by Pavlov using dogs showed that pairing a bell tone with food (which automatically elicits a salivation response) could result in the bell tone becoming associated with the food. Subsequently, the bell tone elicits salivation without the food (Pavlov, 2003). Since these experiments, associative learning studies have used the classical conditioning paradigm to show that pairing a neutral stimulus (conditioned stimulus; CS) with an aversive or a rewarding stimulus (unconditioned stimulus; US) can result in the CS eliciting similar responses (conditioned response; CR) to those that are elicited by the US (unconditioned response; UR; Gormezano, Kehoe, & Marshall, 1983). These studies have been instrumental in demonstrating how seemingly unrelated stimuli develop the ability to elicit similar responses. There is some debate within the classical conditioning literature between theories of stimulus substitution and stimulus response (Gormezano, Kehoe, & Marshall, 1983). Although discussion of that debate is beyond the scope of the current thesis, it represents an important consideration for further investigation beyond the current work.

1.2.3 Related to the imprecision hypothesis

The theoretical application of classical conditioning to a pain event seems intuitive. A painful event constitutes a stimulus package. The imprecision hypothesis postulates that the nociceptive component of that stimulus acts as an unconditioned stimulus (US) and the other components act as a conditioned stimulus (CS). In classical conditioning terms, the sensory input or contextual cue would constitute the neutral CS, while the nociception would constitute the US. Following pairing of the CS and US, and through associative learning processes, the CS can develop to elicit pain. Pain is now considered the conditioned response (CR). This example of stimulus pairing, where pain is the CR, is a departure from conventional conditioning research and theory that involve pain. Fear conditioning studies often use a painful stimulus as the US

and investigate pain-related fear or fear of pain-related movement (Harvie et al., 2016; Karos, Meulders, & Vlaeyen, 2015; Williams & Rhudy, 2007). Therefore, pain, rather than nociception, is usually considered the US. The other departure that this line of inquiry makes from conventional conditioning studies is that seemingly unrelated sensory inputs or contextual cues can elicit a powerfully aversive response such as pain. That is, movements or tactile inputs which are non-nociceptive inputs can become capable of eliciting pain (Moseley & Vlaeyen, 2015).

1.2.4 A closer look at classical conditioning

There is an extensive body of literature on classical conditioning, and the field is characterised by vigorous debates about theory, research and investigation (Rescorla & Wagner, 1972; Klopf, 1988; Öhman & Mineka, 2001; Young, Joseph, & Gray, 1993). With regards to the current thesis, several issues are of particular importance. (1) The magnitude of the CR is often less than that of the UR. Therefore, the CR can be considered as a response that prepares the individual for a consequence (i.e. a preparatory response; Rescorla & Wagner, 1972). (2) When a second CS is added to an existing CS-US pair, the second CS fails to become capable of eliciting the CR (Klopf, 1988). This is thought to occur because no new information can be learnt from the second (new) CS. This process is referred to as *blocking* (Klopf, 1988). For example, if an individual became sick (UR) each time she ate mushrooms (1st CS). Then if she, on another occasion, started to eat mushrooms (1st CS) with spinach (2nd CS) and still became sick. Then the spinach is unlikely to evoke the CR (becoming sick). This is because of the previously-established association between eating mushrooms and being sick (Rescorla & Wagner, 1972). (3) The more ecologically valid the stimulus, the more effective the conditioning will be (Domjan, 2005). For example, classical conditioning studies on fear (Öhman & Mineka, 2001, 2003) have shown that a greater fear response is elicited by a CS such as an image of a snake than by an image of a mushroom or flower. (4) Associative learning is delayed when a familiar stimulus is used, as opposed to when a novel stimulus is used. This process is referred to as *latent inhibition* (Young, Joseph, & Gray, 1993). This is thought to be protective, because the individual is prevented from acquiring inappropriate associations. For example, if we consider Pavlov's experiments, if the dogs had already heard bell tones often, but without them being paired with food, then pairing the bell tones with food would take longer to induce a CR, because the tones had previously

been established as irrelevant stimuli that predicted nothing (Lubow & Moore, 1959). The importance of assessing and investigating the role of both "contingency awareness" and "expectancy awareness" in classical conditioning studies is paramount. A participant is considered "contingency aware" if they can articulate the relationship between a specific CS and a specific US (Lovibond & Shanks, 2002). For example, "The fearful face appears before the painful shock" (specific CS; specific US). "Expectancy awareness", on the other hand, is defined as the awareness of the impending delivery of a specific US, and is triggered by the presentation of a specific CS (Lovibond & Shanks, 2002). For example, a participant's awareness that a painful shock is impending is triggered by the presentation of a fearful face. Although these two constructs seem similar, they are in fact distinct (Lovibond & Shanks, 2002). A participant could report that they expected the US but be unable to articulate why. In that case, the participant would be "expectancy aware" but not "contingency aware". For the participant to be considered both "contingency aware" and "expectancy aware", they would need to explain their expectancy rating by articulating the association between the specific CS and specific US. For example, the participant would need to articulate that they strongly expect the painful shock when they see the fearful face (expectancy aware), *because* the fearful face usually precedes the painful shock (contingency aware).

1.2.5 Differential classical conditioning

Over the decades, classical conditioning has evolved from a simple investigation of *associative learning* between a CS and US, to the use of different CSs to investigate *differential learning* (Rescorla, 2014). In the differential classical conditioning paradigm, one CS is paired with an aversive US (danger signal; CS+) and another CS is paired with either a lower intensity US or is unpaired (safety signal; CS-; Pearce, 1987). The use of two CSs allows researchers to observe whether differential learning has occurred, by examining the difference between responses at a within-subject level. Differential learning is demonstrated when a greater CR is elicited by a CS+ than by a CS- (Vlaeyen, 2015).

1.2.6 Stimulus generalisation within a classical conditioning context

Stimulus generalisation is an extension of the learning acquired through classical conditioning (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015). It occurs when acquired learning is extrapolated to other similar, yet novel, stimuli that share functional, physical or perceptual features with the original CS. These stimuli are called generalisation stimuli (GS; see Figure 3) (Vlaeyen & Linton, 2012). The learnt association between two stimuli allows the individual to predict potential consequences. When it comes to dangerous stimuli, associative learning is vital for survival, because the ability to predict danger, and therefore avoid it, is protective. Recognition of similar stimuli allows the individual to avoid potential danger without having to experience it (Vlaeyen, 2015). This process of recognising potential dangers is commonly referred to as *stimulus generalisation*, whereby the individual recognises a new stimulus as being similar to a known dangerous stimulus and extrapolates the meaning associated with the known dangerous stimulus to the new similar one. The individual then responds to the new stimulus as they would to the known, dangerous stimulus. Importantly, while generalisation helps the individual to recognise positive alarms (true threats), it also increases their risk of responding to false alarms (non-threats; Vlaeyen, 2015).

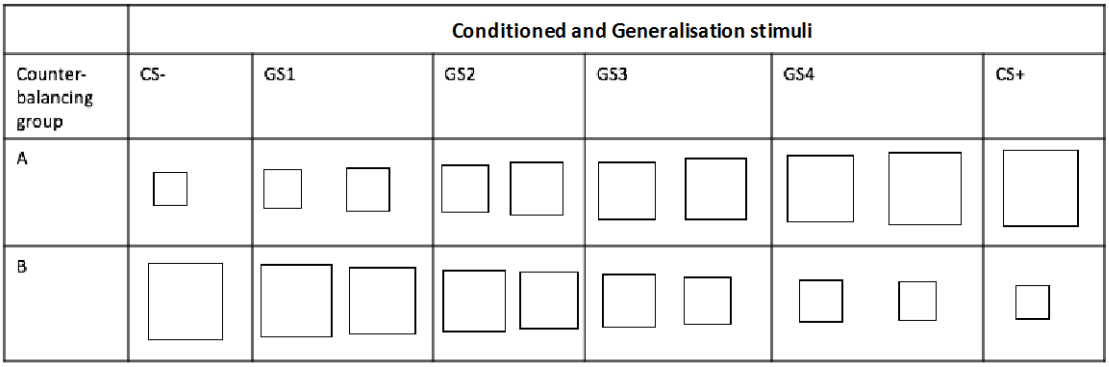


Figure 3. This figure depicts different-sized squares as the generalisation stimuli (GS) and conditioning stimuli (CS). In this figure, the GS range in size between that of the two conditioning stimuli. This figure also shows the counterbalancing of the CS+ and CS-. Group A would receive the largest square, as the CS+ and the smallest as the CS- and Group B would receive the reverse. Image adapted from Lissek et al. (2008).

1.2.7 Formation of a generalisation gradient

The response data from participants in generalisation studies is processed to produce *generalisation gradients* (see Figure 4).

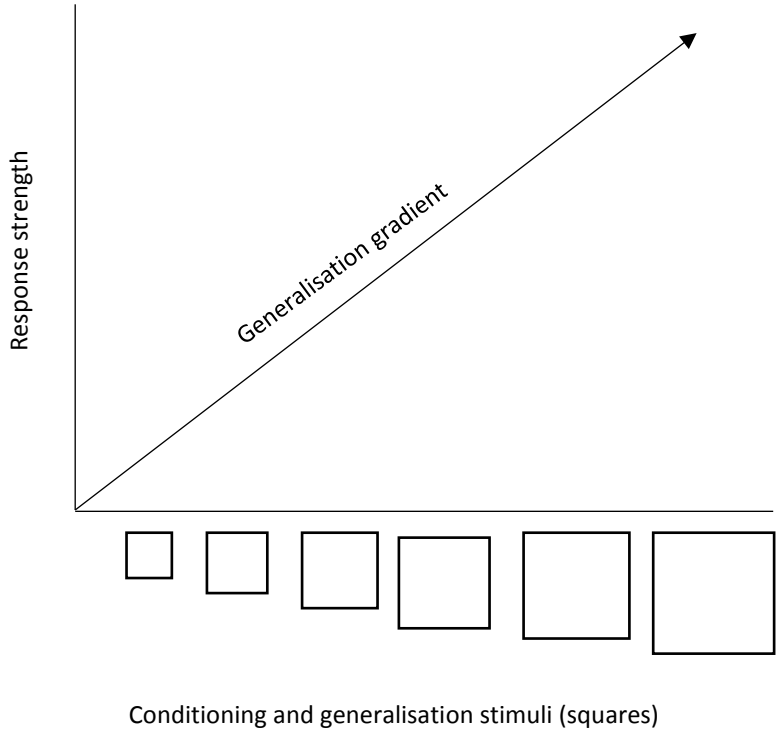


Figure 4. Illustration of the formation of a generalisation gradient. First, the researcher arranges the GSs according to how similar they are, either physically, functionally or perceptually, in relation to the CSs (Struyf et al., 2015). As depicted in this figure, the CSs and GSs are arranged according to similarities in size. Second, each GS and CS is considered as a separate category. The difference in the size of the GS compared to the size of the CS is categorised on the *x*-axis. Third, the response data from all the participants, for each GS and CS, are averaged. That is, the average response strength was calculated for each size of square (*y*-axis). Fourth, the averaged response data are then plotted for each GS and CS. The results are expected to demonstrate a gradient that relates participants' average responses to the stimuli (Struyf et al., 2015). As the size of the GS increases similarly to the size of the CS, the strength of the response is greater, or more similar to the recorded CR to the CS.

1.2.8 Differential generalisation

The use of a CS+ and a CS-, and GSs that range in similarity between the two CSs, enables the researcher to examine generalisation. That is, if the response of interest is greater in the presence of a GS that is similar to the CS+, than in the presence of a GS that is similar to the CS-, then differential generalisation has been demonstrated (Pearce, 1987). If the response is similar in the presence of all stimuli, irrespective of whether the stimuli are more similar to the CS+ or to the CS-, then differential generalisation *has not* been demonstrated. For example, one study (Meulders, Meulders, Stouten, De Bie, & Vlaeyen, 2017) investigated generalisation of conditioned pain-related fear and expectancy of pain-related movement in people with fibromyalgia (FM) and healthy controls. The study paired one movement (CS+) with a painful stimulus and another movement (CS-) was unpaired. Next, the participants were required to complete five novel but similar movements (GSs) which varied in similarity between the CS+ and the CS-. Compared to healthy controls, people with fibromyalgia expected similar pain for all movements, regardless of whether the movement resembled the CS- or the CS+ more closely. Therefore, people with FM *did not* demonstrate differential generalisation of expectancy of pain.

1.2.8 Evidence of differential generalisation of a classically conditioned response

It is well-established that pain-related fear can become a classically conditioned response (Karos et al., 2015; Meulders, Harvie, Moseley, & Vlaeyen, 2015). For example, a study by Meulders et al. (2015) used left/right hand judgements to investigate this issue. Left/right hand judgements involve presenting a participant with an image of a hand, and having them judge whether it is a left or right hand. In order to do this, the individual mentally rotates their own hand to match the orientation of the hand posture image. The study by Meulders et al. (2015) paired one left/right hand judgement with a painful electrocutaneous stimulus (CS+), while another left/right hand judgement was unpaired (CS-). Participants rated their fear of the pain associated with the CS+ higher, than of the non-pain-associated CS-. This demonstrates differential learning. In addition, this classically conditioned pain-related fear generalised to other similar, yet novel, left-right judgement hand postures. That is, this study showed that, as the left-right judgment hand postures increased in similarity to the CS+, the conditioned pain-related fear response increased.

1.2.9 Problematic assumptions of stimulus generalisation studies

There are two assumptions underlying stimulus generalisation that are problematic. The first is that participants are able to distinguish between stimuli selected by the researcher and the second is that participants would intuitively arrange stimuli in the same order as the researcher would (Struyf et al., 2015; Vervliet & Van, 2015). The first assumption—that an individual is able to distinguish between the CS and a novel but similar GS—is a problem that most generalisation studies need to assess. Therefore, most studies have tested whether individuals can distinguish between the proposed CS and GS. That an individual recognises a difference between the threatening CS and the novel GS is of more importance if the GS and CS used are more ecologically valid (e.g. movements; Zaman et al., 2015). This is because, if the participants have distinguished between the stimuli, then the recorded responses would accurately reflect stimulus generalisation. That is, the individual has recognised that the novel GS is similar to, but distinct from, the CS which predicted the aversive US, yet concludes that the GS may also predict the US. Hence, the individual responds to the novel GS in a similar way.

The second assumption—that participants intuitively rank stimuli similarly to the researcher—is perhaps reasonable in the case of studies such as Lissek et al. (2008), where the stimuli are images of circles. However, the assumption's reasonableness is perhaps less clear when more functional stimuli (e.g. faces or movements) are used for ecological validity (Zaman et al., 2015, 2016). In this case, individual idiosyncrasies may play a role in the ranking of the stimuli. For example, participants with persistent back pain may rank the different back movements from the most to least painful, whereas others may group them according to the most flexed position to the most extended position. These differences, if not assessed, could affect the interpretation of the response data used to test for generalisation (Zaman et al., 2015).

Related research questions: Do participants sequence GSs in the same way and using the same criteria as researchers?

Chapter 4, '*Placing stimuli in order: conflicts between the choices of researchers and participants*' investigates this question as it relates to the paradigms used in this thesis and the relevant literature.

Related research question: Do participants accurately discriminate between GSs?

Chapter 6, '*Are these faces different? Perceptual acuity for different faces - an electronic survey*' investigates this question as it relates to the paradigms used in this thesis and the relevant literature.

1.3 Modulation of pain by non-somatosensory factors

Pain is a response that can be manipulated in various ways, such as by altering participants' expectations, providing different verbal instructions, or through observation to alter the threat value of the situation (Vlaeyen, 2015). Perhaps the most extensive base of literature on modulating threat in the context of pain is the work on nocebo and placebo effects (Tracey, 2010). The majority of studies in this area have shown that expectation influences the reported pain experience (Enck, Benedetti, & Schedlowski, 2008; Jamshidian, Hubbard, & Jewell, 2014; Salomons et al., 2004). If participants expect less pain, then the reported pain intensity and associated neural processes of the aversive stimulus are diminished (placebo; Tracey, 2010). However, if participants expect more pain, then the opposite occurs (nocebo; Tracey, 2010). However, there is some controversy within the placebo and nocebo literature about how consistent this seemingly directional effect actually is (Enck et al., 2008).

Several studies (Arntz & Claassens, 2004; Moseley & Arntz, 2007; Wiech et al., 2010) have effectively illustrated how modulation of threat influences the reported pain experience. A study by Arntz and Claassens (2004) that applied a cold metal bar to participant' necks, showed that manipulation of verbal information about the temperature of the stimulus modulated the reported pain. The study used two groups of participants; one group was told that the bar was hot, and the other was told that it was cold. The majority of participants in the "hot" group (13 out of 16) reported the bar being more painful than the median pain rating. Meanwhile, the majority of participants in the "cold" group (14 out of 16) reported the bar being less painful than the median rating. A study by Moseley and Arntz (2007) used red and blue lights to manipulate the meaning associated with a cold (-20°C) metal bar. Red light was assumed to alter the meaning of the stimulus—that it would be more tissue-damaging—and blue light was assumed to alter the meaning of the stimulus as less tissue-damaging. Moseley and Arntz (2007) showed that participants rated the same cold metal bar as hotter, with more unpleasant and intense pain, when paired with the red light than with the blue light. Moreover, participants rated the stimulus as more unpleasant if the red light preceded the delivery of the cold metal bar than if it occurred at the same time as the stimulus delivery. In a similar vein, Wiech et al. (2010) used threat manipulation to investigate how the perceived threat value of an imminent stimulus altered reported pain. This was done in two stages; first, participants were told that

the researchers needed to assess the safety of laser stimulation to different areas of the skin – this was a sham skin examination intended to manipulate threat. Some skin areas were identified as "approved" areas to stimulate (low threat) and other skin areas were identified as "approved with caution" (high threat). Second, participants' pain-threshold level (the point at which a stimulus begins to be perceived as painful) was calibrated. Participants then received laser stimuli to the different areas of the skin previously identified as "approved" or "approved with caution". Before each stimulation block, participants were informed as to whether the stimulation would be delivered to the "approved" skin area (low threat) or the "approved with caution" skin area (high threat). Wiech et al. (2010) found that identical laser stimuli were more likely to be rated as painful during the high threat manipulation than during the low threat manipulation.

1.3.1 Evidence for conditioned pain

While there is evidence that pain can be a response, there is no evidence that demonstrates pain can be a conditioned response to a CS alone. However, there is limited and conflicting evidence that a non-painful stimulus can become painful (termed *allodynia*) due to a classical conditioning effect. A systematic review (Madden et al., 2016) and two subsequent experimental studies (Madden et al., 2016; Madden, Russek, Harvie, Vlaeyen, & Moseley, 2016), investigated the idea of conditioning-induced allodynia. The systematic review (Madden et al., 2016) was specific, in that only studies that had used conditioning without verbal suggestions were included. This was done so as to prevent the influence that verbal suggestions may have on participants' pain perceptions. That is, if a verbal suggestion alluded to a change in pain, then participants may expect and therefore report changes in pain. A total of three studies were included and analysed. This systematic review found that the evidence demonstrating that classical conditioning *alone* can be used to elicit pain to a stimulus that is usually non-painful is inconclusive. This finding is intriguing, as most clinicians think that there is strong evidence that pain is a classically conditioned response (Madden & Moseley, 2016).

The two experimental studies (Madden, Bellan, et al., 2016; Madden, Russek, et al., 2016), conducted after the systematic review, investigated whether classical conditioning could be

used to elicit pain. Once again, conflicting results were found for conditioning-induced allodynia. The first study (Madden, Russek, et al., 2016) used a differential classical conditioning paradigm to investigate whether participants' pain thresholds changed depending on which CS a heat stimulus was paired with. Classical conditioning did not show a shift in participants' heat pain thresholds. The second study (Madden, Bellan, et al., 2016) also investigated whether pain could be elicited due to a classical conditioning effect. This study used a differential classical conditioning paradigm to investigate whether participants' pain thresholds changed depending on which conditioning stimulus a laser stimulus was paired with. It was found that, after the classical conditioning procedure, more at-pain-threshold stimuli were reported as painful when paired with the CS+ than when paired with the CS-. This study demonstrated a shift in pain thresholds, from non-painful to painful, due to a classical conditioning effect

1.3.2 Evidence for conditioning-induced hyperalgesia.

While evidence for conditioning-induced allodynia is conflicting and limited, evidence for conditioning-induced hyperalgesia (when a painful stimulus is perceived as more painful than normal) is more clear. The same systematic review (Madden, Harvie, et al., 2016) identified ten studies that investigated whether classical conditioning could be used to make an already-painful stimulus more painful ("conditioning-induced hyperalgesia" or "conditioned pain amplification"). Of the ten studies, eight showed hyperalgesia as a classically conditioned response. While these studies (Madden et al., 2016) reported conditioning-induced hyperalgesia, and other studies (Meulders et al., 2015; 2017; Meulders & Vlaeyen, 2013b) have shown generalisation of conditioned pain-related fear or fear of movement-related pain, only one study (Harvie et al., 2016) has investigated generalisation of conditioned hyperalgesia. This study failed to show generalisation of conditioning-induced hyperalgesia. However, there are two limitations to consider regarding this study which hinders its findings. First, the researchers' main outcome was conditioned fear, and generalisation of conditioning-induced hyperalgesia was an exploratory outcome. Second, the study only investigated the generalisation of conditioning-induced hyperalgesia *after* the participants had gone through a testing phase investigating conditioned fear. During that fear-testing phase, the GSs were not reinforced: participants did not receive a painful stimulus with the GS. Therefore, the non-reinforced fear-testing phase may have extinguished any classically conditioning effect on pain, which could

explain the null results (Harvie, Meulders, Reid, et al., 2016). Although the study showed no evidence for the generalisation of conditioning-induced hyperalgesia, responses may have been extinguished before testing for them, hence limiting the ability to draw any conclusions.

Accordingly, this thesis aimed to address this gap in the literature by determining whether conditioning-induced hyperalgesia can be elicited in the presence of similar but novel stimuli, using a differential classical conditioning paradigm. In order to minimise the limitations of the study by Harvie, Meulders, Reid, et al. (2016) this study investigated the possibility of generalisation of conditioned hyperalgesia as an *a priori* primary aim. The current thesis investigated this possibility in two ways. First, the study in Chapter 5 was based on similar studies by Meulders et al. (2015) and Meulders and Vlaeyen (2013a) that showed *generalisation* of pain-related fear and expectancy of the painful US due to a conditioned effect. Second, the study in Chapter 7 was based on similar study by Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar (2015) that showed *conditioning-induced hyperalgesia* to faces. Investigating the possibility of generalisation of conditioning-induced hyperalgesia using two approaches was deemed necessary. This is because of the different theories (sometimes conflicting) regarding the different experimental components of classical conditioning, such as the type of CS, and the timing of the CS and US, that can impact on the conditioned effect. Therefore, this thesis used similar studies that have shown either generalisation of a conditioned response (in the case of Chapter 5) or conditioning-induced hyperalgesia (in the case of Chapter 7) to inform parts of the study design.

Related research question: Does conditioned hyperalgesia decrease as the similarity of the generalisation stimuli (hand posture images) to the CS+ (highly painful hand posture image) decreases?

Chapter 5, titled '*Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?*' addresses this question.

Related research question: Does the conditioned response of hyperalgesia decrease as the similarity of the generalisation stimuli (face images) to the CS+ (highly painful face image) decreases?

Chapter 7, titled *'Healthy controls show conditioned generalisation of hyperalgesia to face images'*, addresses this question.

1.4 Summary of components of the imprecision hypothesis of chronic pain that are addressed in this thesis.

The imprecision hypothesis (Moseley & Vlaeyen, 2015) has recently reignited discussion about the potential role of associative learning in persistent pain. Moseley and Vlaeyen (2015) hypothesised that, if researchers were to pair a non-noxious stimulus (CS) and a nociceptive, pain-eliciting stimulus (US), then the CS may cause or contribute to pain as a conditioned response (CR). This example of pairing, where pain is the CR, has two unique features. Firstly, nociception can be used as the US, and secondly, a seemingly unrelated non-noxious stimulus can elicit a powerfully aversive response such as pain (Moseley & Vlaeyen, 2015). This chapter has explained aspects of the theoretical framework for the development of the imprecision hypothesis that, until now, remain to be clarified. The following chapters report both the methods developed and the research questions asked, in order to investigate the assumptions of the imprecision hypothesis regarding perceptual discrimination and generalisation. An overview of these chapters is given below.

- Chapter 2 – *Methods* – presents the development of all the experimental procedures for the studies contained in each chapter.

- Chapter 3 - *Pain's sensory and affective qualities – differentially modifiable dimensions or two aspects of a unitary experience? A systematic review.* This chapter addresses the *pain dimension focus* component of the imprecision hypothesis through a systematic review of the literature (see Figure 5).

- Chapter 4 – *Placing stimuli in order: conflicts between the choices of researchers and participants.* This chapter investigates whether the researcher's ranking of the eight hand stimuli is the same as that chosen by the respondents. This is an extension of the perceptual discrimination literature to ask a question is particularly relevant when investigating stimulus generalisation. Few studies have investigated the ranking of experimental stimuli in the context of pain. This chapter addresses the *perceptual discrimination* component of the imprecision hypothesis (see Figure 5).

- Chapter 5 – *Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?* This chapter investigates whether the conditioned response of hyperalgesia decreases as the similarity of the generalisation stimuli (hand posture images) to the CS+ (highly painful hand posture image) decreases. This chapter addresses the *imprecision and generalisation* component of the imprecision hypothesis (see Figure 5).

- Chapter 6 – *Are these faces different? Perceptual acuity for different faces - an electronic survey.* This chapter investigates whether respondents can distinguish between different face images. This chapter addresses the *encoding and imprecision* component of the imprecision hypothesis (see Figure 5).

- Chapter 7 – *Healthy controls show conditioned generalisation of hyperalgesia to face images.* This chapter investigates whether the conditioned response of hyperalgesia decreases as the similarity of the generalisation stimuli (face images) to the CS+ (highly painful face image) decreases. This chapter addresses *the imprecision and generalisation* component of the imprecision hypothesis (see Figure 5).

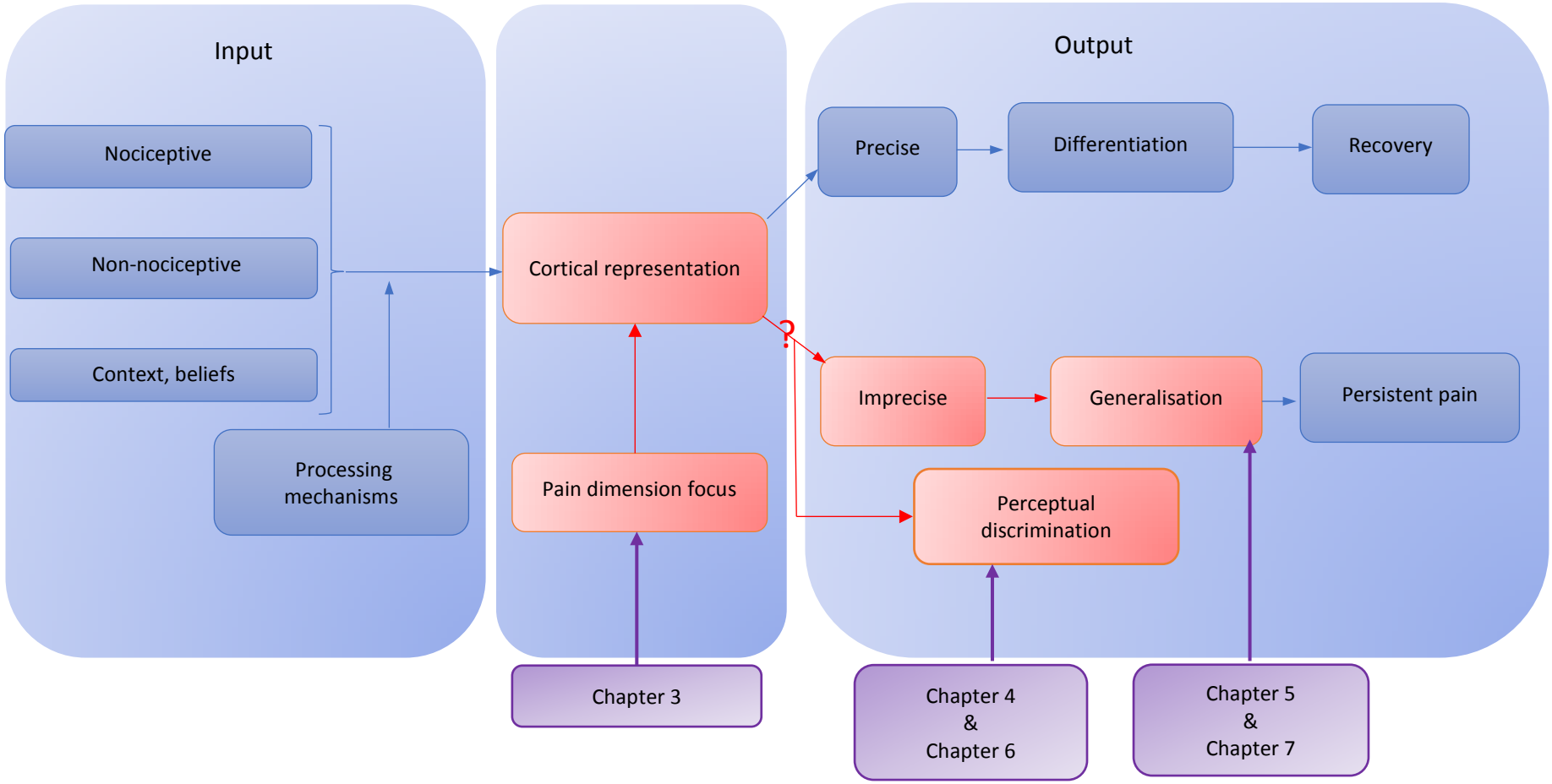


Figure 5. Outline of the concepts of the imprecision hypothesis of chronic pain. The red boxes represent the components investigated by this thesis.

2

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

2. Methods

The experiments of the current thesis required the development or refinement of new methods, and extensive piloting. This chapter focuses on the methods that addressed these issues.

Section 2.1 presents the importance of transparency and posting of a priori *protocols.*

Section 2.2 presents the development and piloting of surveys to test aspects of perceptual discrimination.

Section 2.3 presents the development and piloting of a calibration procedure for use with the classical conditioning studies contained in Chapters 5 and 7.

Section 2.4 describes the programming of experimental procedures using different software packages.

Section 2.5 presents the development of the conditioning and generalisation stimuli for the classical conditioning studies contained in Chapters 5 and 7.

Section 2.6 presents the piloting process used for the classical conditioning studies contained in Chapters 5 and 7.

Section 2.7 presents the development of a method used to assess perceptual discrimination for the study reported in Chapter 7: "Healthy controls show conditioned generalisation of hyperalgesia to face images".

2.1 Transparency

There are three fundamental principles that are deemed important in research: transparency, openness and reproducibility. While many researchers claim to embrace these principles, there is limited evidence for many researchers actually implementing procedures to ensure they are achieved (Nosek et al., 2015). A focus of my training has been the integration of these principles into my research.

Due to this focus, all the study protocols and analysis plans for the work presented in this thesis were pre-registered and unforeseen deviations from the protocols have been fully disclosed in the manuscripts. The following pre-registered protocols are available in the appendices:

Appendix 1 presents the protocol developed *a priori* for the systematic review contained in Chapter 3: *Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review*.

Appendix 2 presents the protocol developed *a priori* for the survey contained in Chapter 4: *Sequencing of conditioning and generalisation hand stimuli, the same or different? An electronic survey*.

Appendixes 3 and 4 present the protocol developed *a priori* for the study contained in Chapter 5: *Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?* This protocol was posted on the Open Science Framework (OSF) and was originally posted as two separated experimental protocols. However, it has subsequently been presented as one study.

Appendix 5 presents the protocol developed *a priori* for the study contained in Chapter 7: *Healthy controls show conditioned generalisation of hyperalgesia to face images* (posted on OSF).

Appendix 6 presents the protocol developed *a priori* for the calibration procedure used in the study contained in Chapter 7.

Before commencing piloting or posting the protocols, each protocol was reviewed by at least three reviewers. Protocols for the classical conditioning studies reported in Chapters 5 and 7 were also reviewed by an independent member of the research group based at Neuroscience Research Australia.

2.2 Development and piloting of the surveys

As highlighted previously in the introductory chapter, ensuring the validity of the stimulus sequence, and that the stimuli are distinguishable from each other, is of paramount importance (Vervliet & Van, 2015). Therefore, two surveys were developed to enable the researchers to investigate different aspects of perceptual discrimination.

2.2.1 Development of the surveys

Both surveys took the form of electronic surveys (e-surveys) administered on the internet, and were developed using the SurveyMonkey platform. A development checklist (Table 1) was devised from the *Checklist for Reporting Results of Internet E-surveys* (CHERRIES) to ensure both surveys met important criteria for reporting (Eysenbach, 2004).

Table 1. Checklist for development of the e-surveys presented in this thesis.

Survey Topic	Item
Recruitment	Identify target population for survey
Recruitment process	Administered through the internet only. Identified social media platforms (Facebook, Twitter). Advertisement design and development for the different platforms used.
Ethical considerations	Design informed consent process. Identify data collection and storage processes.
Survey type	Voluntary, open survey available for each visitor.
Question types	All questions must be mandatory, with a 'not applicable' response available.
Incentives	No incentives.
Review process	Determine whether the 'back' button would be available.
Access restriction	Use of Internet Protocol (IP) address to prevent one respondent from participating multiple times.
Analysis	Only complete questionnaires would be analysed. Determine statistical analysis of completed questionnaires.

2.2.2 Piloting of the e-surveys

Both e-surveys underwent the same two-stage piloting process.

Stage one occurred after initial development. Three independent reviewers assessed the technical functionality of the e-survey and critiqued the definitions of terms used. Consensus

between the reviewers and the developer was established before the e-survey entered stage two.

Stage two involved the administration of the survey to a small group of respondents, comprising researchers with expertise in the field of interest. Respondents were asked to provide feedback on the usability, timing and functionality of the e-survey. Once the feedback was addressed, the e-survey was then re-piloted on a group of respondents, comprising some of those in the first group and some new reviewers. The respondents included from the first group were asked to check that all the previous concerns had been addressed. The new respondents were asked to provide feedback on the usability, timing and functionality of the e-survey. Once the feedback had been addressed, the e-survey underwent a final review and then was administered to the wider population.

2.3 Development and piloting of the calibration procedure

Identifying the appropriateness of the calibration procedure used to determine the intensity assigned to a specific pain rating is important (Madden et al., 2016). Failure to calibrate the correct intensity to the required pain rating may compromise the conditioning procedure. The intensity must be aversive enough to ensure that the conditioning is effective (Rescorla & Wagner, 1972).

2.3.1 Piloting of calibration procedure for the study (Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?) contained in Chapter 5

A Neodymium: Yttrium-Aluminum-Perovskite (Nd: YAP) laser (1340 nm wavelength) was used in this study. During piloting, two parameters were tested: (1) spot size (4 mm, 5 mm and 6 mm) and (2) pulse duration (5 ms and 6 ms). This was done to determine the most effective stimulus, while complying with published recommendations for safety (Madden, Catley, et al., 2016).

A single ascending staircase design was piloted. That is, participants were given a sequence of stimuli of gradually increasing energy, starting from the lowest setting on the machine. This determined whether a single ascending staircase achieved the target pain intensity ratings of 3/10 and 5-6/10, which were required for this study. Moreover, it identified the ranges in which the pain intensity ratings were achieved. Once the target pain intensity ratings had been achieved, the researchers piloted how consistent the target rating was and what to do if it became inconsistent. Therefore, two parameters were tested:

I. The number of repeated laser stimulations that should be delivered at different locations on the skin but at the same intensity (3 or 6).

II. The proposed increment by which the laser intensity (0.25 J or 0.50 J) should be adjusted if the target rating was found to be inconsistent.

If two of the three repeated stimulations, *or* four of the six repeated stimulations were rated at the target rating, then the laser stimulus percept was considered consistent and the chosen laser energy retained. If more than two of three or four of six of the ratings of the

repeated stimulations exceeded the target intensity rating, then the laser energy was decreased by either 0.25 J or 0.50 J. If 75% of the ratings of the repeated stimulations were below the target intensity rating, then the laser energy was increased by either 0.25 J or 0.50 J.

The CNC Mac 3 software program (Solid Edge ST 8, Australia) controlled and moved a robotic arm to which the laser hand piece was attached. This device was used to ensure that no two successive laser stimuli were delivered to the same skin location. The software receives programmed coordinates at which the laser hand piece was located for each trial. In order to determine the optimal distance that the laser hand piece needed to move to ensure no overlapping of stimulation sites, different co-ordinate options were developed and piloted. The coordinates piloted were 5 mm, 8 mm and 10 mm apart. The time taken for the robotic arm to move the laser hand piece to the next stimulation site was measured for each distance. This measurement helped ascertain the time needed between each trial (intertrial interval; ITI).

Once the optimal distance was established, the two different movement patterns of the robotic arm were piloted. The two patterns tested were a zigzag pattern and an up-across-down pattern (Figure 1).

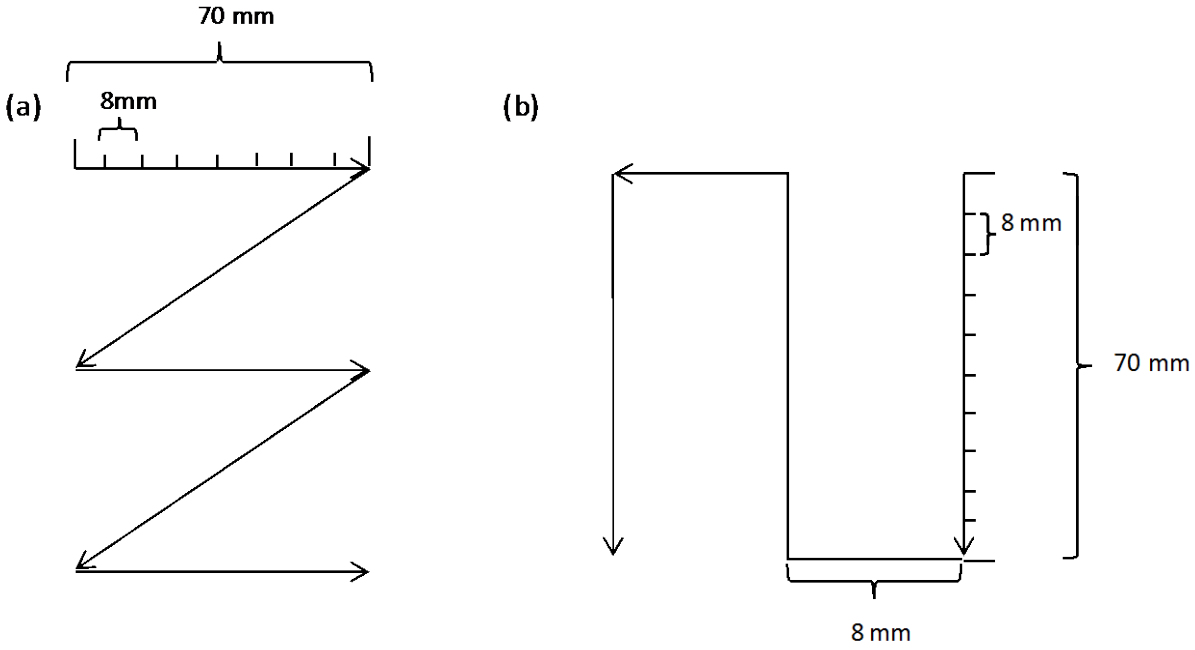


Figure 1. The two robotic arm stimulation patterns that were piloted. Image (a) illustrates the zigzag movement pattern and image (b) illustratrates the up-across-down movement pattern. The robot arm moves 8 mm between each stimulation. Once the robot arm reaches 70 mm the robot arm changes the direction.

Once the movement pattern of the robotic arm had been determined, two additional parameters were established: (1) the area that would be mapped out on the dorsum of the right hand and, (2) the end-point coordinates (to ensure that the robotic arm moved the laser hand piece within the mapped-out area on the hand and did not move onto the fingers or forearm). Once determined, the optimal coordinates and pattern were entered into the CNC Mac 3 program.

2.3.4 Piloting of calibration procedure for the study contained in Chapter 7 (Healthy controls show conditioned generalisation of hyperalgesia to face images).

A calibration protocol was developed and tested for use (Appendix 6). Electrocutaneous stimulation was employed as the US in this study. A practice phase was developed and tested to identify the stimulus intensity that would allow the participant to become accustomed to the stimulus and limit potential influencers, such as anxiety, that could exaggerate pain intensity ratings during calibration.

A single ascending staircase method was used. Several different step-wise progressions (mA) and inter-pulse intervals (sec) were piloted (Table 2).

Table 2. Piloting of different step wise progressions and inter-pulse intervals.

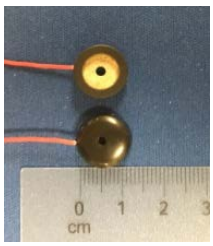
Electrical stimulus intensity (mA)	**Step-wise progression (mA)**	**Inter-pulse interval (sec)**
Start: 2.0	1.0, 1.5	30, 40, 50-60
Once 9.0 reached	1.5, 2.0, 2.5	30, 40, 50-60
Once painful (intensity variable)	2.0, 3.0 and 4.0	30, 40, 50-60

Three different pain ratings were required for this study (a non-painful US, a moderately-painful US and a highly-painful US). Different types of electrodes were tested to determine the best type/s to deliver the three different intensities. The following electrodes (Figure 2) were tested:

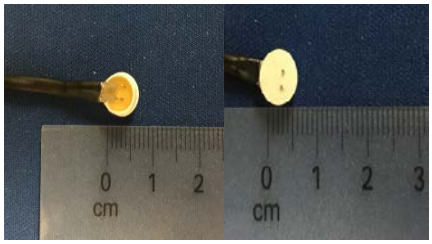
I. transcutaneous electrodes (consisting of a separate anode and cathode),
II. intracutaneous electrodes (which were designed in-house at the University of South Australia), and
III. cardiac lead electrodes.

Image/s

(a)



(b)



(c)

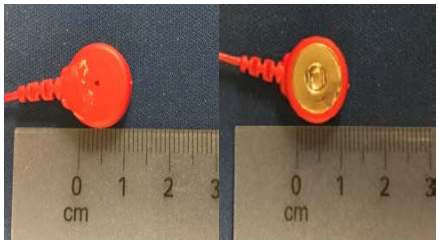


Figure 2. Images of the different electrodes piloted for Study Two. Image (a) is of the transcutanoues electrodes, image (b) is of the intracutaneous electrodes and image (c) is of the cardiac lead electrodes used.

The range in which the non-painful stimulus became painful, and the perceived quality of the stimulus, were recorded. During piloting, participants received the stimulation through the different electrodes and were asked to rate and describe the quality of the different intensities.

2.4 Programming of the experimental procedures

Each experimental block of the study contained in Chapter 5 (*Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?*) was pre-programmed using Gentask software (Compumedics Neuroscan, El Paseo, Texas). The Gentask software controlled the delivery of the images (displayed on the participants' screen) and laser stimuli. The order, delivery and timing of the images were piloted to determine the optimal process for the experiment. In addition, the timing of the delivery of the laser stimulation using transistor-to-transistor logic (TTL) signalling was piloted. This was done to ensure that the laser stimulus was delivered correctly. For example, the laser stimulus was delivered 300 ms after the CS+ image in the Acquisition phase. Pilot testing on participants started once the initial experimental procedure had been established and programmed in Gentask.

Each experimental block of the study contained in Chapter 7 (*Healthy controls show conditioned generalisation of hyperalgesia to face images*) was pre-programmed using Affect 4.0 software (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2009). The Affect software controlled the following features of the experiment:

I. The delivery and semi-randomisation of the images, following a rule that the CS+ and CS- could not be displayed consecutively.
II. All participant responses were recorded in an output file.
III. The different stimuli were delivered via transistor-to-transistor logic signalling.
IV. The intensity of the modality delivered was specified according to the trial type programmed by the experimenter.
V. The timing of each different experimental trial flow.
VI. Any restrictions specified by the experimenter. Specifically, the button-push option was restricted to allow participants to push the button only eight times during certain phases of the experiment.
VII. The display options on both the participants' and researchers' screens.
VIII. The different rating scales used were programmed, as well as the method by which the participants would record their rating (a mouse click for pain intensity and US expectancy, and a push of a keyboard space bar for the button option).

Each experimental phase was developed and programmed, and the flow, timing and recording of responses were checked. Additionally, the required intensities (low, moderate or high), and the timing and delivery of the electrocutanous stimuli were manually checked. Once the experimental procedure was programmed, the experiment was fully automated.

2.5 development of the conditioning and generalisation stimuli

The conditioning and generalisation stimuli used in the study contained in Chapter 5 (*Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?*) were adapted from Meulders et al. (2015). The hand posture images were created from photographs of a right hand in front of a black background. Emphasis was placed on ensuring that the angle of the wrist remained the same. This was achieved by measuring the wrist angle using a goniometer. The images showed the same hand in postures that varied from fully flexed to fully extended. The proposed sequence in which the hand postures would be placed to form a generalisation gradient for the Chapter 5 study was assessed in the Chapter 4 study (Figure 3).

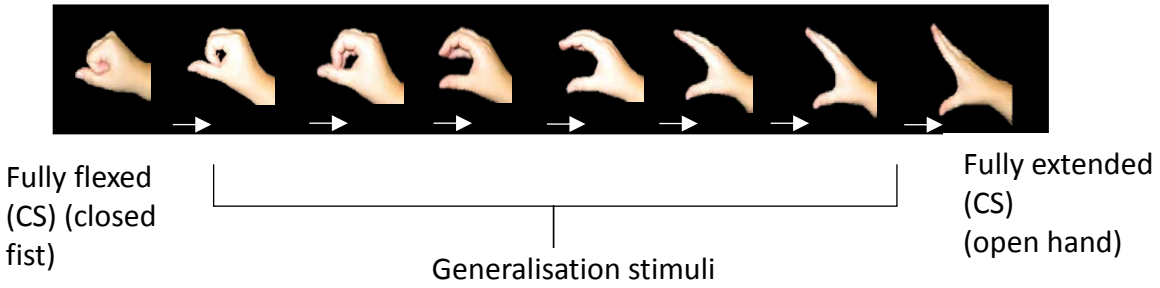


Figure 3. The sequence of hand images used as conditioning and generalisation stimuli in the study *Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?* The fully flexed and fully extended hand posture images were used as conditioned stimuli (CS) and the hand postures that ranged in similarity between the CS were used as generalisation stimuli.

Distractor stimuli (DS) were also developed using the same method described previously. The distractor stimuli were images of hand postures that were distinctly different to those used for the CS and GS.

The conditioning stimuli used in the Chapter 7 study (*Healthy controls show conditioned generalisation of hyperalgesia to face images*) were sourced from the Karolinska Directed Emotional Faces (KDEF) set (www.emotionlab.se/resources/kdef). This set of 4900 images was developed for use in perception, attention, emotion, memory and masking experiments. The set consists of pictures of 70 individuals (35 female, 35 male; mean age 25 years; age range 20—30) with seven different facial expressions per individual (Lundqvist, Flykt, & Öhman, 1998). After obtaining permission for their use, five pairs of distinct faces, with

neutral facial expressions, were identified to be used as conditioning stimuli (CS). Generalisation stimuli (GS) for each distinct face pair were created using the Face Film (version 3.3.20; Yan 2016) application run on a Macbook Air computer. This application allowed facial elements to be combined to create images that varied along a continuum between the two distinct faces (Figure 4).

After development, the GS underwent four stages of rigorous piloting to ensure that the final GSs used in the experiment were perceptually distinct. In **Stage One**, four reviewers were sent the five sets of proposed CSs and GSs. Each independently reviewed the CS and GS sets and rated them from 1 to 5, where 1 indicated their first choice for use in the experiment as the CS and GS, and 5 indicated their last choice. The face set that was most strongly recommended was chosen. In **Stage Two**, the chosen CSs and GSs were further piloted via an e-survey (Chapter 6) to determine the distinguishability between the faces.

The remaining face stimuli were chosen following analysis of data from this e-survey. In **Stage Three**, the chosen face stimuli were programmed into the Affect software and a group of participants completed the entire experiment. The pain intensity ratings and US expectancy ratings were analysed, and the results used to further refine the selection of face stimuli. Face stimuli for which all participants rated both the US expectancy and pain intensity the same were deemed to be indistinguishable from each other and were removed. In **Stage Four**, the remaining face stimuli were re-piloted and the results checked using the same exclusion criteria.

Distractor stimuli used for this study were images of distinct male faces with neutral facial expressions. These were also sourced from the KDEF set (www.emotionlab.se/resources/kdef) (Lundqvist et al., 1998).



Figure 4. The face images used as conditioning and generalisation stimuli in the Chapter 7 study.

2.6 The piloting process used in the two classical conditioning studies.

Once the components of the experiment (e.g. the GS and CS, or the timing of the electrocutaneous stimulus) had been piloted, refined and checked, the entire experimental procedure for each study was piloted. Figure 5 presents a general overview of the experimental procedure for Study One and Study Two.

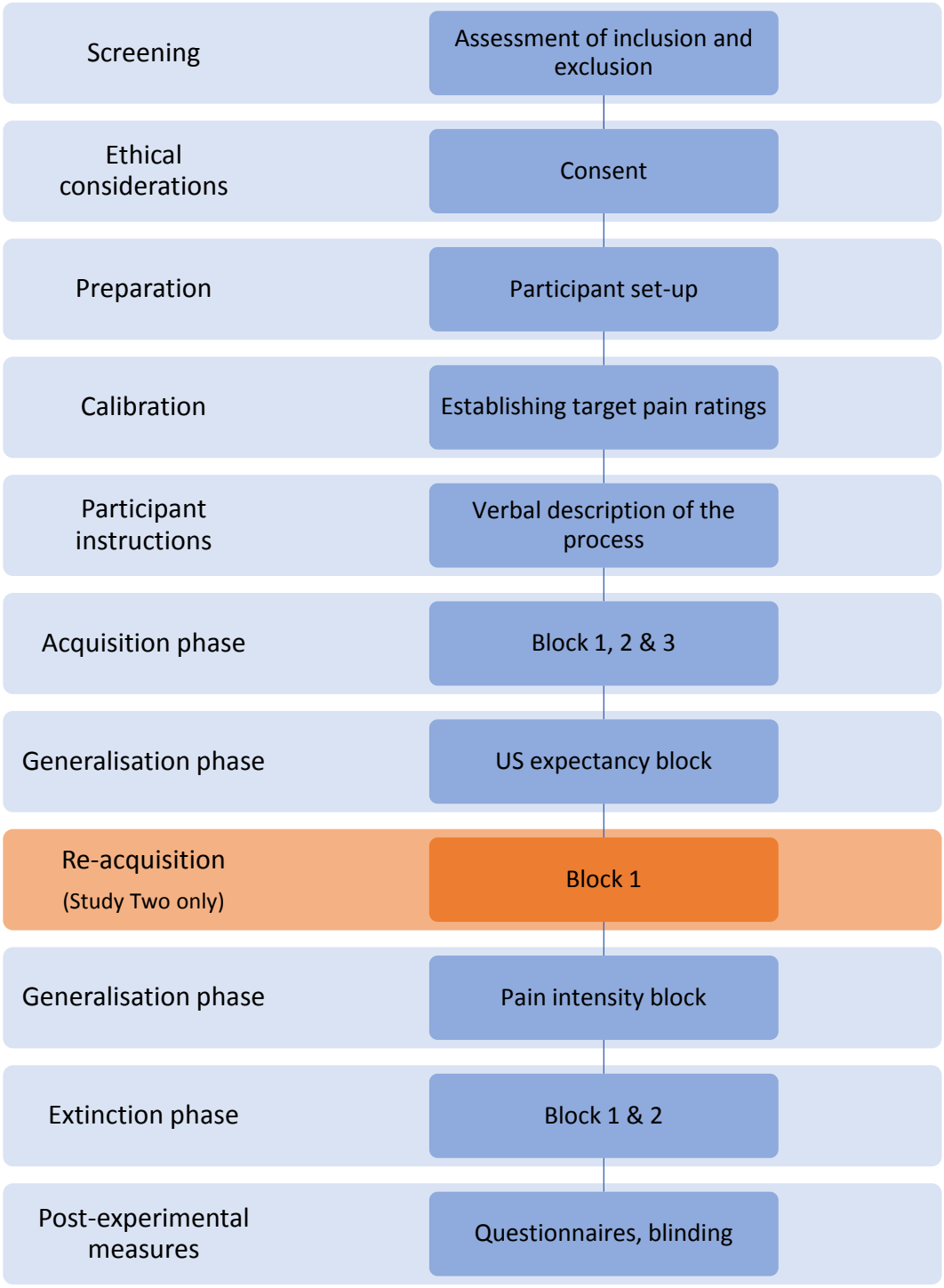


Figure 5. General overview of the classical conditioning experimental procedure that was piloted for Studies One and Two.

The Chapter 5 study (*Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?*) was piloted to check the following components of the experimental procedure:

I. That the time period between the image disappearing and the delivery of the US was sufficient for providing a verbal rating of US expectancy and pain intensity.

II. The clarity of the questions displayed on the screen.

III. The clarity of the participant instructions.

IV. Whether the time allocated between trials was sufficient to allow the robotic arm to move to the next stimulation location.

V. Whether the mapped-out area on the dorsum of the hand was accurate for different hand sizes.

VI. The time taken to complete the entire experiment.

This minimised potential errors that could affect the results, and ascertained the feasibility of the rating scales and times allotted for rating. Any problems identified, either through the formal assessments or noted in feedback from participants, were adjusted and then re-piloted.

The Chapter 7 study (*Healthy controls show conditioned generalisation of hyperalgesia to face images*) was piloted to allow for analysis of the following components of the experimental procedure:

I. The timing of the trial flow for each experimental block.

II. That all the CS and GS are displayed the correct number of times.

III. The clarity of both the verbal and written instructions provided to the participants.

IV. Ease of use of the system used by participants to rate US expectancy and pain intensity.

V. The average time taken for participants to provide each rating.

VI. That the correct stimulus was delivered with the correct CS, GS or DS, and at the correct time (e.g. the US expectancy question must be displayed before delivery of the US).

VII. Whether the limits set on the button option programmed for this experiment:

a. would interrupt only highly painful stimuli,

b. stopped interrupting a highly painful stimulus if pushed more than eight times.

VIII. The output file where all the participants' responses would be recorded was checked.

IX. The timing of the entire experiment.

This minimised any potential errors that could affect the results of the study. If the timing allocated to rate both US expectancy and pain intensity was not sufficient, then data would be lost unnecessarily.

2.7 Development of a method to assess perceptual discrimination in the Chapter 7 study

To assess perceptual discrimination between the face images used in this study, a two-alternative, forced-choice paradigm was developed. After the experiment, participants were shown a series of faces that had been used in the experiment. Participants were allocated, *a priori*, to one of four randomised blocks of face image pairs (randomisation undertaken in Excel). Participants were tasked with identifying the two face images as identical or different to one another, in order to assess perceptual discrimination. Each block included all the face images used in the study, but in a different order, to reduce the risk of an order-effect. Each face image was compared to all the other face images. For example, the CS+ face image was compared to each GS face image and to the CS- face image. Images within a pair were displayed sequentially (Figure 6). Following display of the face-image pair, the participant was prompted to judge whether the two faces were 'the same' or 'different'. Each block was then piloted to determine the optimal delay between display of each image. Delays of 5 and 3 sec were trialled. This was to establish whether the delay allowed enough time to view an image and then rate it. The optimal delay between the display of image pairs and questions was also assessed. In addition, participants were asked for feedback on the clarity of the instructions provided in order to minimise any misunderstandings that could affect the results.

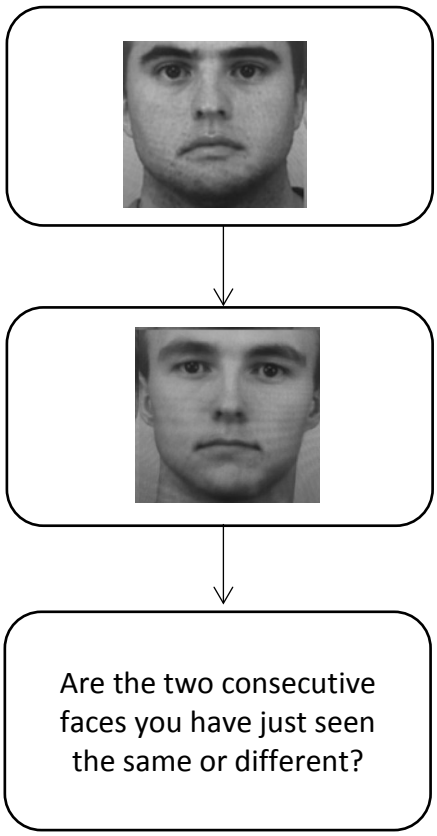


Figure 6. Sequential presentation of the facial images and question used in the perceptual discrimination task.

Introduction

Methods

3 Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

3. Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

The imprecision hypothesis of chronic pain suggests that a heightened affective state may increase the imprecision with which a painful event is encoded and represented in the brain, resulting in a pain response that is no longer elicited in the presence of specific stimuli. This underlying assumption is driven by research into post-traumatic stress disorder (PTSD) and generalised anxiety disorder. Individuals with these conditions have difficulty in responding to specific threatening stimuli, but instead respond to stimuli that are less and less like the original threatening stimuli.

That pain has both sensory and affective dimensions is not recent knowledge. Research has shown that certain pain conditions can be differentially influenced by each dimension. Certainly, clinical evidence has shown that people with persistent pain more readily express the affective dimension of pain than the sensory dimension. However, whether these dimensions are differentially modifiable is contentious. Therefore, in order to be able to determine the role of heightened affect in the imprecision hypothesis of chronic pain, first one must determine whether the sensory and affective dimensions are, in fact, differentially-modifiable dimensions.

The following review has been submitted for publication and is formatted accordingly. It begins on the next page.

Title: Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

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Abstract

It is recognised that pain has both a sensory quality (“How intense is it?”) and an affective quality (“How unpleasant is it?”). Although pain feels like a single unpleasant bodily experience, investigations of human pain have long considered these two dimensions of pain to be separable and differentially modifiable. Remarkably, the evidence underpinning this separation is seldom presented. We discuss the current evidence for whether or not the sensory and affective dimensions of pain can be selectively modulated using cognitive manipulations. A rigorous systematic search of the literature, conforming to recommendations and based on *a priori* search terms and consultation with experts in the field, yielded 4270 relevant articles. Following an in-depth critique of the twelve articles that met the inclusion criteria, we found a lack of compelling evidence that the sensory and affective dimensions of pain can be selectively and intentionally modulated in humans. We offer potential explanations for this discrepancy and contend that this surprising finding highlights several important questions for the field, from both research and clinical perspectives.

Keywords

Sensory dimension, Affective dimension, Dimensions of pain, Selective modulation, Cognitive manipulations

Main Text

Introduction

Pain commonly drives its sufferer toward safety; it can be considered a motivator of protective behaviour rather than a marker of tissue damage (Moseley & Butler, 2015; Moseley & Vlaeyen, 2015; Wallwork, Butler, Wilson, & Moseley, 2012). The clear and inherent motivational aspect of pain makes it more closely resemble feelings such as hunger or thirst than sensory perceptions such as seeing or hearing. That pain is necessarily perceived – one cannot have pain and not know about it – and its inherent unpleasantness (or indeed its juxtaposition to pleasure) have occupied human thought for millennia. Plato wrote "…All pleasure and pain come to be in the soul, because all pleasure and pain is necessarily perceived ..." (*Philebus* 33c-34c, interpretation by Erginel, 2011). Towards the end of the last century, this immutable emotional nature of pain was recognised more formally as one dimension of a bidimensional experience that has both a sensory-discriminative dimension and an affective-motivational dimension (Melzack, 1975, 1987; Price et al., 1987).

The sensory-discriminative dimension, often referred to simply as 'intensity' (Auvray, Myin, & Spence, 2010) or sometimes given the label 'sensory', includes the spatial and temporal characteristics of pain: where it is felt, whether it is sharp or dull, deep or superficial, burning, aching, or stinging. The affective-motivational dimension (Melzack, 1975) is often referred to simply as 'unpleasantness' (Fledman, 2009; Melzack, Wall, & Ty, 1982; Price, Bush, Long, & Harkips, 1994; Price & Dubner, 1977) or is given the label 'affective', and does not involve spatial, temporal or qualitative characteristics. It describes how bad or unpleasant the pain is. That is, it describes the motivational aspect of pain – the aspect that makes us want to take protective action (Moseley & Butler, 2015). The notion that people can recognise that pain is both sensory and affective led to the development of the most widespread and tested pain assessment tool - the McGill Pain Questionnaire (Melzack, 1975, 1987), which includes a long list of descriptors that are categorised as sensory or affective, and is used by clinical psychologists and other pain-related professionals the world over. There is a large body of literature concerning the properties of the McGill Pain Questionnaire and the recognisable patterns of responses that can allow differential diagnoses. For

example, those diagnosed with neuropathic pain were more likely to endorse sensory descriptors than affective ones, and those with neuropathic pain were less likely to endorse affective descriptors than those with non-neuropathic pain (Boureau, Doubrere, & Luu, 1990).

This bi-dimensionality of pain has also been corroborated by clinical reports (Dubuisson & Melzack, 1976; Price et al., 1987; Taenzer, Melzack, & Jeans, 1986; Wade, Price, Hamer, Schwartz, & Hart, 1990). Several studies have reported that the relative contributions of the sensory and affective dimensions are related to perceived threat. That is, those who perceive their pain to be associated with a greater threat to their health will rate the pain's unpleasantness higher than those who perceive their pain to be associated with a lesser threat to their health, despite having similar self-reported pain intensities (Price et al., 1987; Rainville et al., 1992). Price et al. (1987) observed that the pain intensity reported during childbirth was higher, but the pain unpleasantness was lower, than the pain reported in some chronic pain states or in cancer-related pain. Similarly, the sensory qualities of the pain associated with heartburn are consistent whether the sufferer attributes their pain to heartburn or to myocardial infarction (a 'heart attack'), but the affective qualities of the pain differ – those who attribute their pain to a heart attack rate the affective dimension more highly than those who attribute it (correctly) to heartburn (Melzack & Casey, 1968; Melzack, Taenzer, Feldman, & Kinch, 1981; Price et al., 1987).

Cognitive manipulations during brain imaging have been used to tease apart the cortical activation patterns associated with the sensory and affective dimensions of pain (Maihofner, Herzner, & Handwerker, 2006; Rainville, 2002; Rainville et al., 1999). Experiments implicate the anterior cingulate cortex (ACC) in subserving pain unpleasantness in particular. For example, a cognitive manipulation to increase the affective dimension of pain (i.e. to make it more unpleasant but not more 'intense') reportedly caused an increase in ACC activation. A cognitive manipulation to decrease the affective dimension (i.e. make it less unpleasant but no less intense) reportedly caused a decrease in ACC activation (Rainville et al., 1997). Similar direction-specific modulation of the primary somatosensory cortex (S1) was reported in conjunction with manipulation of the sensory dimension of pain (Emmert et al., 2014; Hofbauer, 2001; Rainville et al., 1997; Rainville et al., 1992). These studies have been used to support the idea that the sensory and affective dimensions of pain are in fact distinct, and are able to be modulated endogenously—or by the individual's own efforts—independently

of one another (Fledman, 2009; Horn, Blischke, Kunz, & Lautenbacher, 2012; Stohler & Kowalski, 1999). This idea is of critical importance to clinical psychology because it follows that interventions might be best focussed on dampening the affective, rather than the sensory, dimension of pain.

Functional neuroanatomical data have also been used to substantiate the claim that these two dimensions of pain involve distinct neural pathways from the dorsal horn of the spinal cord to cortical centres (Hofbauer, Rainville, Duncan, & Bushnell, 2001; Price, 2000; Treede, Kenshalo, Gracely, & Jones, 1999). These neural pathways have distinctive features that are consistent with selective roles in subserving the affective or sensory dimensions (Treede, Apkarian, Bromm, Greenspan, & Lenz, 2000). The lateral spinothalamic pathway receives synaptic input that is highly dependent on primary nociceptive afferent neurons that originate in laminae I and V-VI of the dorsal horn (Price, 2002). Its main cerebral projection is to the ventroposterior lateral (VPL) nucleus of the thalamus, which then transmits nociceptive input to the primary (S1) and secondary (S2) somatosensory cortices (Willis, Zhang, Honda, & Giesler, 2001, 2002). Given that the primary function of S1 and S2 is sensory processing, it seems reasonable to propose that the lateral spinothalamic pathway is a distinct neural pathway that subserves the sensory dimension of pain (Peyron, Laurent, & Garcia-Larrea, 2000). Strengthening this proposition is the finding that nociceptive neurons within the spinothalamic pathway are predominantly wide dynamic range (WDR) neurons – they respond to a wide range of stimulus intensities (Treede et al., 2000). Several studies have identified that more than 80% of neurons that can be identified as nociceptive are indeed WDR (and the remaining 20% are nociception-specific; NS; Lee, Dougherty, Antezana, & Lenz, 1999). This is important here, because WDR neurons are, by definition, responsive to a wide range of stimuli, from light touch to heavy stimulation, enabling different responses to minutely different stimulus intensities (Coghill, Mayer, & Price, 1992). Clearly, this response profile strongly implies that the spinothalamic pathway is critical and highly precise in encoding the intensity characteristics of a noxious stimulus. It follows then, that this pathway would be particularly important in subserving a sensory dimension of pain.

The spino-parabrachio-amygdaloid and spino-parabrachio-hypothalamic pathways are thought to be integral for the affective dimension of pain (Price, 2002). This view is mainly based on two observations. First, the pathways are composed mostly of NS neurons (neurons which are exclusively responsive to nociceptive stimulation). Second, the central

projections for these two pathways terminate in the amygdala and hypothalamus (which form part of the so-called limbic system; Rainville, 2002). The central nucleus of the amygdala is believed to play a role in fear, emotional memory and behaviour, and autonomic and somatomotor responses to threatening stimuli—functions also thought to be subserved by hypothalamic nuclei (Rainville, 2002). Indeed, given the central targets of the spino-parabrachio-amygdaloid and spino-parabrachio-hypothalamic pathways, it is reasonable to suggest that they subserve the affective dimension of pain (Price, 2002). It is interesting to note that spinothalamic pathways to more ventral and medial thalamic nuclei, such as the ventromedial portion of posterior nuclear complex (VMpo) and the ventrocaudal portion of the mediodorsal nucleus (MDvc), also have greater proportions of NS neurons than WDR neurons, and the VMpo and MDvc are greatly modulated by attentional and motivational states (Willis & Westlund, 1997). As such, VMpo and MDvc neurons have also been suggested to predominantly serve the affective dimension of pain (Price, 2002).

While most neurophysiologists and neuroanatomists seem accepting of these ideas, other specialists have argued that pain is in fact a unidimensional construct, and that separating it into dimensions is as fallacious as attributing it to single neurons (Smart & Doody, 2006). It has also been argued that the apparently successful attempts to independently modulate the two dimensions of pain via cognitive strategies—for example, in the imaging studies mentioned earlier (Emmert et al., 2014; Hofbauer et al., 2001; Rainville et al., 1997; Rainville et al., 1992)—actually reflect demand effects (or the effects of 'coaching' participants), rather than selective endogenous modulation of pain's two dimensions (Chapman et al., 2001; Fernandez & Turk, 1994). Indeed, whether or not people can even differentiate the two dimensions in real time remains contentious (Chapman, 1996; Chapman et al., 2001, 2002). Several studies have shown that participants tend to rate the sensory and affective dimensions of pain similarly, unless they receive clear instructions that explicitly highlight differences between them (Chapman et al., 2001; Fernandez & Turk, 1994). The most common instructional technique is metaphorical, for example "…you think of listening to a sound, such as a radio. As the volume of the sound increases, I can ask you how loud it sounds or how unpleasant it is to hear it. The intensity of pain is like loudness; the unpleasantness of pain depends not only on intensity but also on other factors which may affect you…" (Price, McGrath, Rafii, & Buckingham, 1983, p. 47). However, whether or not it is possible to differentially monitor and modulate the two dimensions is relatively

unimportant according to Chapman et al. (2001), who surmised that, because people are not consciously aware of two dimensions of pain unless they are explicitly instructed to differentially modulate them, the distinction between them is clinically uninformative. An alternative view, however, is that selective reduction of the affective dimension of pain could offer profound clinical benefit, because it would reduce the unpleasantness of pain without compromising the potentially important stimulus-relevant information offered by pain's sensory dimension.

The notion that pain has two separable dimensions which can be independently and endogenously modulated is widely accepted by some (predominantly scientists; Fledman, 2009; Rainville, 2002; Rainville et al., 1992), and soundly rejected by others (predominantly clinicians, philosophers and lay people; Younger, McCue, & Mackey, 2009). Despite this, there seems to have been very little attempt to evaluate the actual *evidence* that the sensory and affective dimensions of pain can be differentially targeted and modulated. This is surprising, because experimental studies continue to differentiate them, whereas clinical practice continues to consider pain as a single construct. Patients are almost always asked 'How is your pain?' rather than 'How unpleasant is your pain?' and 'How intense is your pain?' Within the context of the lived experience of pain and the position it takes within social interactions, we might ask 'How bad is it?', or 'Where is it?', but in such interactions, we are considering pain as a unitary, unpleasant sensory experience which affects *that* person. 'If someone has a pain in his hand, then the hand does not say so … one does not comfort the hand, but the sufferer: one looks into his face' (Wittgenstein & Von Wright, 1998, p. 127). Therefore, by failing to evaluate the actual *evidence* for whether the separation and independent modulation of the sensory and affective dimensions of pain is possible, existing studies, clinical interactions and treatments based on either viewpoint remain questionable. That is, if we can truly modulate one dimension without the other, then there seems to be merit in the idea of developing cognitive treatments by which sufferers could make their pain less unpleasant but no less informative.

We aimed to fill this substantial and surprising gap in the knowledge base by systematically evaluating the evidence for the notion that the two dimensions of pain can be selectively modulated in humans using a cognitive strategy. We undertook a systematic review based on a protocol determined *a priori*, and a standardised appraisal of experimental bias. Our approach was informed by recommendations made in a landmark review of this field

undertaken over two decades ago (Fernandez & Turk, 1992), which remains the most recent review on this topic. That work concluded that, in order to address the contention that threatened to polarise the field, there needed to be (i) a comprehensive evaluation of the effectiveness of attempts to modulate the two postulated dimensions of pain, (ii) a structured framework and standardised appraisal to examine the methodological rigour of each study, including potential threats to the validity of uncontrolled biases, with each included study having an *a priori* intention to use a cognitive strategy to selectively modulate one of the two dimensions of pain, and (iii) careful planning so that all such investigations are planned according to sound theoretical reasoning.

Objective

To determine the extent to which the affective and sensory dimensions of pain can be selectively and intentionally modulated by cognitive manipulations in people with chronic pain and in healthy volunteers.

Method

This systematic review was carried out following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher, Liberati, Tetzlaff, & Altman, 2009) and an *a priori* protocol based on the recommendations of Fernandez and Turk (1992; see Appendix A).

Information sources

The following databases were searched for articles dating from their inception through to February 2017: the Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library; MEDLINE (OvidSP); SciVerse SCOPUS; PsycINFO (OvidSP); Embase Classic and Embase (OvidSP). A sensitive search strategy was devised that used a combination of keywords and relevant subject headings. The aim was to identify studies that investigated the selective cognitive modulation of the sensory and affective dimensions of pain. The initial search strategy (see Appendix B) was developed in Medline (OvidSP), and then adapted for other databases. In an effort to detect all relevant studies, content experts were also contacted and asked to identify any additional published or unpublished material that

was relevant to the review question. Reference lists of all eligible studies, key textbooks and previous reviews were also hand-searched for further records.

Study selection

The titles and abstracts of all records that were identified by the initial search were independently screened and evaluated, using a predetermined list of inclusion criteria, by two reviewers (KAT & VJM). Studies were included if they met all of the following criteria: tested human participants; applied a cognitive technique with the aim of selectively modulating either the sensory or the affective dimension of pain; followed an experimental study design that had at least one of the following: measured a baseline, had a control group, had a pre-post design; assessed both the sensory and affective dimensions. Studies were excluded if they used somatosensory (e.g. transcutaneous electrical nerve stimulation) or exogenous pharmacological interventions (e.g. morphine) to modulate pain. Studies could include participants with acute pain, chronic pain, or no pain, in which case the study would involve experimentally-induced pain. Where more than one participant group was included, the groups were evaluated separately. The search process was not limited by language, but the titles and abstracts had to be in English to allow initial assessment. Full texts were retrieved for all eligible records, and independently appraised by two reviewers (KAT & VJM). Non-English full-text articles that were identified as potentially eligible were translated by two independent translators before assessment.

Outcome measures

The primary outcomes were participant ratings of the sensory and affective dimensions of experiences that were evoked by painful stimuli. The secondary outcomes were differences in cortical activation, physiological responses and psychological variables associated with the manipulations.

Risk of bias across studies

We developed, tested and refined a customised risk-of-bias tool (see Appendix C). This tool was then independently applied to all selected studies by two reviewers (KAT and VJM). For each category of risk, the reviewers highlighted particular factors that could be sources of bias. Minimum criteria were defined for a low risk-of-bias rating in certain categories. Studies were expected to have confirmed the efficacy of their cognitive manipulations by reporting the results of the manipulation check and reporting the efficacy of participant

blinding. Outcome measures were expected to clearly assess pain rather than the stimulus itself. Purposive or convenience sampling regimes were considered to have a high risk of bias (a deliberately conservative decision, although we recognise that, for pragmatic reasons, most research in this field uses convenience sampling).

Data collection process

Data extraction was performed by two independent reviewers (KAT and VJM) using a customised and pre-piloted form (see Appendix D). Extracted data were: 1) participant characteristics such as age and gender, 2) study methods, 3) trial characteristics and 4) details of outcome measures. The results of each study were collated, and the study authors' interpretations of their results, as well as the independent interpretation made by each of the independent reviewers (KAT and VJM), were recorded on a data extraction form.

Any discrepancies that were identified during the risk of bias assessment and data extraction were discussed. If there was no agreement, then a third, independent reviewer (GLM) was consulted. Any missing information required for risk-of-bias assessment or data extraction was sought from the study authors via email. An initial email was sent, followed by two follow-up emails, two weeks apart. If the information had not been received within six weeks of the first contact, then that information was deemed to be missing, and assessments were performed using the information at hand.

Summary measures

Data were pooled when multiple studies performed comparable manipulations. Where available, we used subject-by-subject data from authors and rescaled the primary outcome data to a 101 point (0—100) numerical rating scale (NRS). The pain intensity scale ranged from 0, equivalent to 'no pain', to 100, equivalent to 'the most intense pain imaginable'. The pain unpleasantness scale ranged from 0, equivalent to 'not at all unpleasant', to 100, equivalent to 'the most unpleasant pain imaginable'. Estimates of change were obtained for each pain dimension by subtracting the ratings given during the baseline phase from those given during the experimental phase. In each study, the cognitive manipulation was intended to alter one dimension of pain without altering the other dimension. We therefore subtracted the change in the 'non-target' dimension from the change in the 'target' dimension, producing a mean difference (with standard error) as an estimate of the manipulation's effectiveness. Means and standard errors were then entered in to Review

Manager software (Version 5.2; The Cochrane Collaboration, 2012) using the generic inverse variance weighting, and pooled using the random effects model.

Results

Study selection

The initial search and removal of duplicates yielded 4708 records. After the screening phase, 232 full-text articles were assessed for eligibility. Two records were reviewed with the help of translators. After assessment of eligibility, 12 articles were included in this systematic review. The details of identification, screening and appraisal are depicted in Figure 1.

-Figure 1 about here-

Study characteristics

The details of the 12 selected studies are provided in Table 1. Various cognitive manipulations were used: five studies used hypnotic induction with suggestion, one used suggestion alone (without hypnotic induction), and four used valence modulation secondary to odour (n = 1) or visual images (n = 3), and two used meditation. Although some manipulations were similar, comparability was limited because even studies that used the same class of manipulation used different methods. For example, although Dahlgren et al. (1995) and Rainville et al. (1997) used hypnotic suggestion, Dahlgren et al. (1995) targeted intensity and unpleasantness using different suggestions, but Rainville et al. (1997) targeted unpleasantness only, using different suggestions to elicit either an increase or a decrease in unpleasantness.

All the selected studies used healthy, pain-free, adult participants. That is, there were no studies that included people with acute or chronic pain and also satisfied our *a priori* criteria. All selected studies had biased eligibility criteria. All five hypnosis studies selectively recruited participants with certain susceptibility profiles: four (Dahlgren, Kurtz, Strube, & Malone, 1995; Hofbauer et al., 2001; Rainville et al., 1997, 1999) recruited participants who had moderate to high scores of susceptibility; one (Valentini, Betti, Hu, & Aglioti, 2013) recruited two groups - one group of people who had high susceptibility scores and another group with low susceptibility scores. Both meditation studies (Grant & Rainville, 2009;

Perlman, Salomons, Davidson, & Lutz, 2010) recruited a group of expert meditators who had spent a set number of hours in meditative practice, and a group of novices. Several studies included only participants who had demonstrated successful attentional or emotional modulation of pain during recruitment (Hofbauer et al., 2001; Kunz, Lautenbacher, LeBlanc, & Rainville, 2012; Villemure, Slotnick, & Bushnell, 2003). Overall, there were more female participants (*n* = 164) than male participants (*n* = 148). Two studies (Loggia, Mogil, & Bushnell, 2008; Valentini et al., 2013) recruited only female participants.

-Table 1 about here-

Risk of bias within studies

The collated results of the risk-of-bias assessments are presented in Table 2. All studies either performed poorly in assessments of selection bias or performance and detection bias, or failed to provide sufficient data for risk-of-bias to be assessed. The most common reasons for studies being at 'high risk' of selection bias were due to their use of selective recruitment or convenience sampling. High risk-of-bias in the performance and detection section was caused by a lack of evidence that participant blinding had been successful.

-Table 2 about here-

Six studies (Dahlgren et al., 1995; Kenntner-Mabiala, Andreatta, Wieser, Mühlberger, & Pauli, 2008; Kenntner-Mabiala, Weyers, & Pauli, 2007; Kunz et al., 2012; Loggia et al., 2008; Villemure et al., 2003) scored 'low risk' in the cognitive technique section because they had incorporated a manipulation check for their intervention. Two of those studies (Kenntner-Mabiala et al., 2008; Kenntner-Mabiala et al., 2007) had also excluded participants who had failed to attend to the manipulation. One study (Rainville et al., 1997) scored the highest risk-of-bias overall, by scoring either 'high' or 'unclear' for every category. Two studies (Perlman et al., 2010; Rainville et al., 1997) scored 'high risk' for measurement bias because they used self-report scales with anchors that pertained to the stimulus itself, rather than to pain. All studies failed to provide *a priori* sample size calculations. One study (Perlman et al., 2010) scored a high risk of measurement bias because it included participants who never reported the desired pain rating of eight (out of ten) in reaction to the maximum safe stimulus, hence introducing ceiling effects into the results. Another study (Hofbauer et al., 2001) scored high on the risk-of-bias for performance and detection bias and for statistical

methods for analysing both non-painful and painful heat ratings together. One study (Dahlgren et al., 1995) failed to report details of stimulus calibration or stimulus intensity, so that reviewers where unable to determine whether the given stimulus was perceived as painful or not.

Results of individual studies

Hypnosis induction with suggestion

Five studies used hypnotic induction with suggestion. Four of these five studies found that hypnotic suggestion selectively increased or decreased affective dimension ratings in accordance with the type of suggestions given (Hofbauer et al., 2001; Rainville et al., 1999; Rainville et al., 1997; Valentini et al., 2013). However, they were unable to demonstrate the same effect with the sensory dimension ratings. The three studies that also aimed to selectively modulate the sensory dimension of pain found that the affective dimension ratings were similarly influenced by the modulation (Hofbauer et al., 2001; Rainville et al., 1999; Valentini et al., 2013). Interestingly, this finding was not consistent with the reported changes in cortical activation patterns: when hypnotic suggestion targeted the sensory dimension, there was no evidence of selective change in *ratings* of the sensory or affective dimensions of pain, but there was evidence of selective activation of cortical areas. Suggestions that were intended to modulate the sensory dimension of pain selectively altered cortical activation in the somatosensory areas (S1 & S2), whereas suggestions that were intended to modulate the affective dimension of pain selectively altered ACC activation. The fifth hypnosis study (Dahlgren et al., 1995) found that hypnosis with suggestion of analgesia selectively decreased ratings of the sensory dimension, whereas hypnosis with suggestion of relaxation selectively decreased the affective dimension of pain. Another factor that influenced the effect of the selective modulation was the hypnotic susceptibility of the participants. Three of the studies reported that participants with high susceptibility to hypnosis demonstrated greater selective modulation of the two dimensions than participants with low susceptibility (Hofbauer et al., 2001; Rainville et al., 1999; Valentini et al., 2013).

Suggestion alone (without hypnotic induction)

The only study that used non-hypnotic suggestion found that either the sensory or the affective dimension of pain could be selectively modulated, depending on the kind of suggestion that was given (Kunz et al., 2012). Different to the studies of hypnotic induction with suggestion that were mentioned above, a suggestion that aimed to decrease the sensory dimension of pain caused a corresponding decrease in the pain intensity ratings, while the pain unpleasantness ratings remained unchanged. Furthermore, a suggestion that aimed to decrease the affective dimension of pain caused a corresponding decrease in pain unpleasantness ratings, while pain intensity ratings remained unchanged.

Valence modulation using exteroceptive stimuli

Four studies used exteroceptive stimuli to modulate valence, and tested the effect of the modulation of valence on separate dimensions. Three of these four studies found that a shift in valence produced selective modulation of affective dimension ratings (Kenntner-Mabiala et al., 2008; Loggia et al., 2008; Villemure et al., 2003), whereas one study found that it produced simultaneous and similar shifts of both sensory and affective dimension ratings (Kenntner-Mabiala et al., 2007). Three of these valence modulation studies (Kenntner-Mabiala et al., 2008; Kenntner-Mabiala et al., 2007; Villemure et al., 2003) used a manipulation of participants' attention. Two studies used a three-way manipulation, in which participants were tasked with directing their attention 'towards the pictures', 'towards the sensory experience', or 'towards the affective experience' (Kenntner-Mabiala et al., 2007, 2008). The other study used a two-way manipulation in which participants attended to either the heat or an odour (Villemure et al., 2003). Interestingly, in the two studies by Kenntner-Mabiala et al. (2007, 2008) only sensory ratings changed, but the change was elicited by different manipulations in the two studies. In the first study (2007), the effective manipulation was attention to the sensory experience, whereas in the second study (2008), the effective manipulation was attention to the affective experience. Villemure et al. (2003) also found that only sensory ratings changed, with the attention to the heat condition eliciting an increase in ratings of the sensory dimension.

Meditation

Two studies compared the modulatory effects of two meditation techniques; one study focused on attention (Grant & Rainville, 2009), and the other study (Perlman et al., 2010) on acceptance-based meditation (sometimes referred to as *open monitoring*). When performed by experienced meditators, acceptance-based meditation elicited selective change in pain affect in one study (Perlman et al., 2010), but selective change in pain sensation in the other (Grant & Rainville, 2009). This is interesting, because it was hypothesised that acceptance-based meditation would modulate the affective dimension. The findings for the focused attention approach were similarly muddled. Focused attention yielded no changes whatsoever in one study (Perlman et al., 2010), while it yielded an increase in the sensory dimension in the other study, but only in the novice meditators (Grant & Rainville, 2009). The two studies used focused attention in different ways: Perlman et al. (2010) required attention to be focused on a fixation point (a cross), whereas Grant and Rainville (2009) required attention to be focused on the stimulus site.

Full data were obtained for three studies. We did not undertake a meta-analysis because the three studies used different manipulations, which prevented pooling. Instead, the results of individual studies are depicted using forest plots (Figure 2).

-Figure 2 about here-

Discussion

Despite apparent widespread support of the idea that the affective and sensory dimensions of pain can be selectively and intentionally modulated by different cognitive manipulations, this review was the first attempt to systematically review the evidence for or against it using 'gold standard' methodology and attempted meta-analysis. Our results reveal a less clear-cut evidence base than is implied by the popularity of the idea, but they offer some support to both viewpoints. On the one hand, several studies showed selective down-regulation of the affective dimension; although, as is so often the case, the studies that were potentially the most compromised by bias showed the largest effects. On the other hand, many studies seem to refute the idea of a distinct sensory dimension that can be isolated. Ten of the 12 studies that satisfied our inclusion criteria for this review were unable to show a quantifiable

selective modulation of the self-report ratings of the sensory dimension of pain (Grant & Rainville, 2009; Hofbauer et al., 2001; Kenntner-Mabiala et al., 2007, 2008; Loggia et al., 2008; Perlman et al., 2010; Rainville et al., 1997, 1999; Valentini et al., 2013; Villemure et al., 2003). Only two studies, one that used suggestion without hypnotic induction (Kunz et al., 2012) and one that used hypnotic induction with suggestion (Dahlgren et al., 1995), demonstrated selective modulation of both the affective and sensory dimensions of pain (Kunz et al., 2012), but both studies had a very high risk of bias. As such, we conclude that the current evidence does not support the idea that the sensory dimension of pain can be selectively modulated using cognitive manipulations.

Which cognitive manipulation seemed the most effective at selectively modulating the affective dimension of pain? Of the four cognitive manipulations identified, hypnotic induction with suggestion demonstrated the most consistent effects (Dahlgren et al., 1995; Hofbauer et al., 2001; Rainville et al., 1997, 1999; Valentini et al., 2013). Most of the studies involved a suggestion to decrease or increase pain unpleasantness. The scripts they used, however, did not necessarily articulate unpleasantness. For example, "…although you will continue to experience normal sensation, your experience will seem surprisingly more agreeable…surprisingly more comfortable…surprisingly more restful than you might have expected…" (Kiernan, Dane, Phillips, & Price, 1995, p. 61) or "When you feel the stimulation, you can be mindful of its intensity…mindful of its burning, pricking, stinging quality but mostly, you will feel particularly uncomfortable. Almost as if, when you feel the onset of the stimulus, you can feel an onset of discomfort quickly spreading into your hand…" (Rainville et al., 1999, p. 169). One study used a suggestion of relaxation intended to decrease pain unpleasantness (Dahlgren et al., 1995) that was adapted from the Stanford Hypnotic Susceptibility Scale Form C (SHSS), the wording of which is, "…You are relaxed, very relaxed. By letting yourself go you can become even more relaxed. You can reach a state of deeper, more complete relaxation. You are becoming increasingly drowsy and sleepy…" (Weitzenhoffer & Hilgard, 1962, p. 15). That three studies demonstrated that hypnosis without suggestion was ineffective (Hofbauer et al., 2001; Rainville et al., 1997, 1999), strongly implies that it was the suggestion itself that led to selective modulation of the affective dimension. This might appear concerning because it raises the possibility that the effect is simply one of reporting bias. However, two aspects of the literature suggest otherwise. First, the same suggestions were ineffective for the sensory dimension.

Presumably, reporter bias would be equally applicable regardless of dimension. Second, the one study that investigated neurophysiological markers demonstrated a biologically plausible effect on cortical activation (Rainville et al., 1997). During the two control conditions called 'wake control' and 'hypnotic control', Rainville et al. (1997) demonstrated that no modulatory effect was observed in the S1, S2, the insular cortex and the ACC. It was only hypnotic induction *with* suggestion that altered cortical activation. Therefore, it was hypnotic induction *with* specific suggestions about the affective dimension that selectively modulated the pain unpleasantness ratings and caused an alteration in the ACC activation. Similarly, it was hypnotic induction *with* specific suggestions about the sensory dimension that caused an alteration in the S1 activation; however, this did not translate to a selective modulation of pain intensity ratings. This selective activation during hypnotic induction with suggestion demonstrated that the power to alter cortical activation was related specifically to the suggestion, and was not merely a reflection of the participant being under hypnosis.

Although hypnosis with suggestion might seem an effective method to selectively modulate the affective dimension of pain, there is an important caveat here. All five hypnosis studies recruited highly hypnotisable participants. Only 10 – 20% of the population are classified as highly hypnotisable (Hilgard, Weitzenhoffer, Landes, & Moore, 1961; London & Cooper, 1969), which implies that only 10 – 20% of the population might be able to use hypnotic induction with suggestion to selectively modulate the affective dimension of their pain. Moreover, in the one study that included participants with low susceptibility to hypnosis, the effect of the selective modulation was lower (Valentini et al., 2013). Therefore, the generalisability of this finding to the clinical population would seem very limited (London & Cooper, 1969; Shor & Orne, 1963).

Four studies demonstrated that it is probably possible to selectively modulate the affective dimension of pain by using exteroceptive stimuli to modulate the valence of a test stimulus (Kenntner-Mabiala et al., 2007, 2008; Loggia et al., 2008; Villemure et al., 2003). Odours or pictures were used to manipulate mood and thereby the affective dimension of pain (Kenntner-Mabiala et al., 2007, 2008; Loggia et al., 2008; Valentini et al., 2013; Villemure et al., 2003). Other studies manipulated spatial attention by asking participants to focus on the stimulus, or away from the stimulus (Kenntner-Mabiala et al., 2007, 2008; Villemure et al., 2003). The valence modulation studies had a lower risk of bias than the studies of hypnosis

with suggestion, which may be why the former group had smaller effects. However, it is important to note that neither group of studies had effect sizes that would be considered powerful in a clinical context. The average difference in affective dimension ratings induced by hypnotic induction with suggestion was 4.4 units on a 100-unit scale (Rainville et al., 1999); the difference in ratings for the picture-based studies equated to less than 3 units on a 100-unit scale (Loggia et al., 2008). Clearly, regardless of the mechanism of induction, the magnitude of effect is well short of that which might be considered clinically meaningful and, indeed, that which is measurable at an individual level.

That the affective dimension of pain might be modulated using cognitive manipulation has clear clinical relevance—it is the unpleasantness of pain that gives pain its influence on behaviour (Berna et al., 2010; Loeser & Melzack, 1999; Price & Harkins, 1992b; Rainville, 2002)—but why might the affective dimension be selectively modifiable while the sensory dimension is not? We suspect that the answer lies partially in the relative accessibility of the concept of modulating emotions, as compared to the concept of modulating pain intensity, an answer that raises its own difficulties. Throughout the developmental years and even into adulthood, people learn emotional self-regulation; societal expectations are that people behave in a particular manner that demands such skills (Davidson, Jackson, & Kalin, 2000; Gross, 2002; Ochsner & Gross, 2014; Ochsner et al., 2004). Therefore, when participants are asked to modulate the affective dimension of pain—how 'unpleasant' it feels—they may find this concept easy to understand, and perhaps they tap into well-practised strategies for emotional self-regulation (Gross, 1998; Sheppes et al., 2014). In contrast, the sensory dimension may be more difficult to modulate because participants may consider it to reflect an immutable sensory signal. This would make the task of modulating pain intensity *seem* impossible.

Consistent with this line of thinking are data that show that the majority of people conceptualise pain as a marker of tissue damage (Catley, O'Connell, & Moseley, 2013; Moseley, 2003), which it has now been clearly shown not to be (Moseley, 2004, 2007; Moseley & Butler, 2015; Rio et al., 2014; see Butler & Moseley, 2013 for a comprehensive review of experimental and clinical studies). We suspect that the patients and participants in these studies would conceptualise their pain as 'coming from the tissues' rather than resulting from a highly complex evaluative process and a 'best-guess output' of the brain

that reflects the predicted merit of taking protective action (Moseley & Butler, 2015; Tabor, Catley, Gandevia, Thacker, & Moseley, 2013; Tabor et al., 2015, 2017). If our suspicion is correct, it is not unreasonable to suggest that participants interpret the sensory dimension of pain as 'tissue pain' itself, which would render any cognitive attempt to reduce it seemingly pointless. As such, failure to observe the ability to selectively modulate the sensory dimension may, in fact, reflect failure to engage with the idea in the first place.

Mindfulness training is a very popular clinical intervention in which patients consciously attend to their moment-by-moment experience (Shapiro, Carlson, Astin, & Freedman, 2006). The critical component of mindfulness is the attitude that one assumes when attending to the moment, with a positive attitude typically emphasised. In this way, mindfulness involves a form of emotional regulation. Several studies have shown that mindfulness is broadly consistent with the idea that pain unpleasantness is open to cognitive control but pain intensity is not (Shapiro et al., 2006). That is, the sufferer learns skills of acknowledging the pain by curbing a behavioural response to it (Rosenzweig et al., 2010). Seeing as the behavioural response is primarily motivated by pain's unpleasantness and not its sensory characteristics, this approach may well involve selective modulation of the affective dimension of pain. Certainly, a review of the meditation literature by Grant (2014) has proposed many mechanisms by which mindfulness or meditation may alter pain. However, further work is required to interrogate the mechanisms that contribute to the efficacy of mindfulness in reducing pain and to determine whether the reduction in pain is related to a selective down-regulation of the affective dimension.

A closer comparison of the hypnosis studies highlights the potential impact of demand effects on outcomes (Dahlgren et al., 1995; Kunz et al., 2012). Four of the five studies used a hypnotic suggestion that asked participants to either increase or decrease 'pain intensity'. All four studies failed to demonstrate selective modulation of pain intensity ratings. The fifth hypnosis study (Dahlgren et al., 1995) used a hypnotic suggestion of 'analgesia' to decrease pain intensity, and successfully achieved a selective modulation of sensory intensity ratings. It is plausible that the *idea* of analgesia was critical to this effect. The term 'analgesia' is usually used to describe an effect of medication, an agent that participants may understand to be capable of modulating sensory signalling. As such, the term 'analgesia' might possess conceptual validity that 'reducing pain intensity' does not. In other words, a participant's ability to selectively modulate a dimension of pain in response to hypnotic suggestion may

relate to the extent to which the suggestion is cognitively accessible, and logically reasonable, to the participant (Sheppes et al., 2014). Assessing conceptual understanding, expectation and self-efficacy in future studies of this topic might cast light on these possibilities.

Further adding to the complexity of investigating this notion of separable dimensions are the differences between experimental and clinical pain. In fact, the clear distinctions between experimental and clinical pain are often ignored, but they are fundamentally important: experimental participants know that their pain will be short-lived, that they can withdraw at any time, and that there is very little or, most likely, no risk of injury (Price et al., 1987; Price, Harkins, Rafii, & Price, 1986; Wells & Ridner, 2008). In contrast, chronic pain sufferers expect that their pain will be long-lasting, difficult to control, and may be indicative of tissue damage (Hadjistavropoulos & Craig, 1994; Max, 1992; Wade et al., 1990). Given these differences, it is plausible that experimental studies on healthy volunteers may not show differences in the two dimensions of pain because of the safety cues that dominate the context—that the pain is not permanent, that all risks have either been eliminated or minimised, and that participants can withdraw at any point during the experiment—are inherent to the experimental process. In contrast, for clinical populations, these safety cues are often lacking. Indeed, the unpleasantness of pain in experimental settings may be reduced because of this reduced threat and therefore reduced need to motivate protective behaviour (Moseley & Butler, 2015; Moseley & Vlaeyen, 2015). Moreover, participants in experimental pain studies arguably represent a subgroup of the general population—those who willingly volunteer for pain experiments. It is highly plausible that the characteristics that render them willing to volunteer for pain experiments modulate their experiences and affect the mechanisms by which, and the extent to which, cognitive manipulations might modulate their pain.

Several suggestions can be made for future work on the basis of this review. First, experimental studies need to adopt rigorous methodologies so as to avoid demand effects. Second, the risk of bias assessment identified the need to remove ceiling effects and carefully word the verbal anchors in self-report scales so as to avoid ambiguity and limit response bias. Third, both participants and researchers should be blinded to condition, and participants should be naïve to the experimental hypotheses. Remarkably, such issues are

commonly emphasised (Park, Bang, & Cañette, 2008) yet commonly neglected. Fourth, raw data should be retained for longer periods of time to make pooling of data feasible for meta-analyses.

This fourth recommendation reflects the main limitation of the current work—we were able to obtain raw data for only three of the 12 studies. Other limitations that were encountered during this systematic review were: the methodological challenges of the constituent studies and the possibility that our *a priori* selection criteria may have caused us to miss studies that inadvertently invoked selective modulation of separate dimensions of pain. However, the approach used by this systematic review was based on well-endorsed recommendations (Fernandez & Turk, 1992), and its adherance to 'gold standard' methods (by clearly defining inclusion criteria in light of expert consensus) offers us confidence in our findings.

Conclusion

In contrast to popular opinion, evidence that the dimensions of pain can be selectively modulated was only found for the affective dimension. The two cognitive manipulations that appearred to have the strongest evidence and largest effects for selective modulation of the affective dimension were: 1) hypnotic induction with suggestion and 2) valence modulation using exteroceptive stimuli. Critically, however, the threats to the validity of the primary studies, and the small effect sizes of the more rigorous studies, are sufficient to warrant only tentative endorsement. Also in contrast to popular opinion, there is moderate evidence that the sensory dimension of pain cannot be selectively modulated. We are not sure why the affective dimension might be selectively modifiable but the sensory dimension not. Possible explanations include demand effects, previous learning, conceptual and contextual limitations and failure to engage, and fundamental differences between experimental and clinical pain. That there were different findings for the two dimensions suggests that the results are probably not all explained by bias effects. However, this systematic evaluation of the existing literature highlights that several important questions remain unanswered and further experimental work is warranted.

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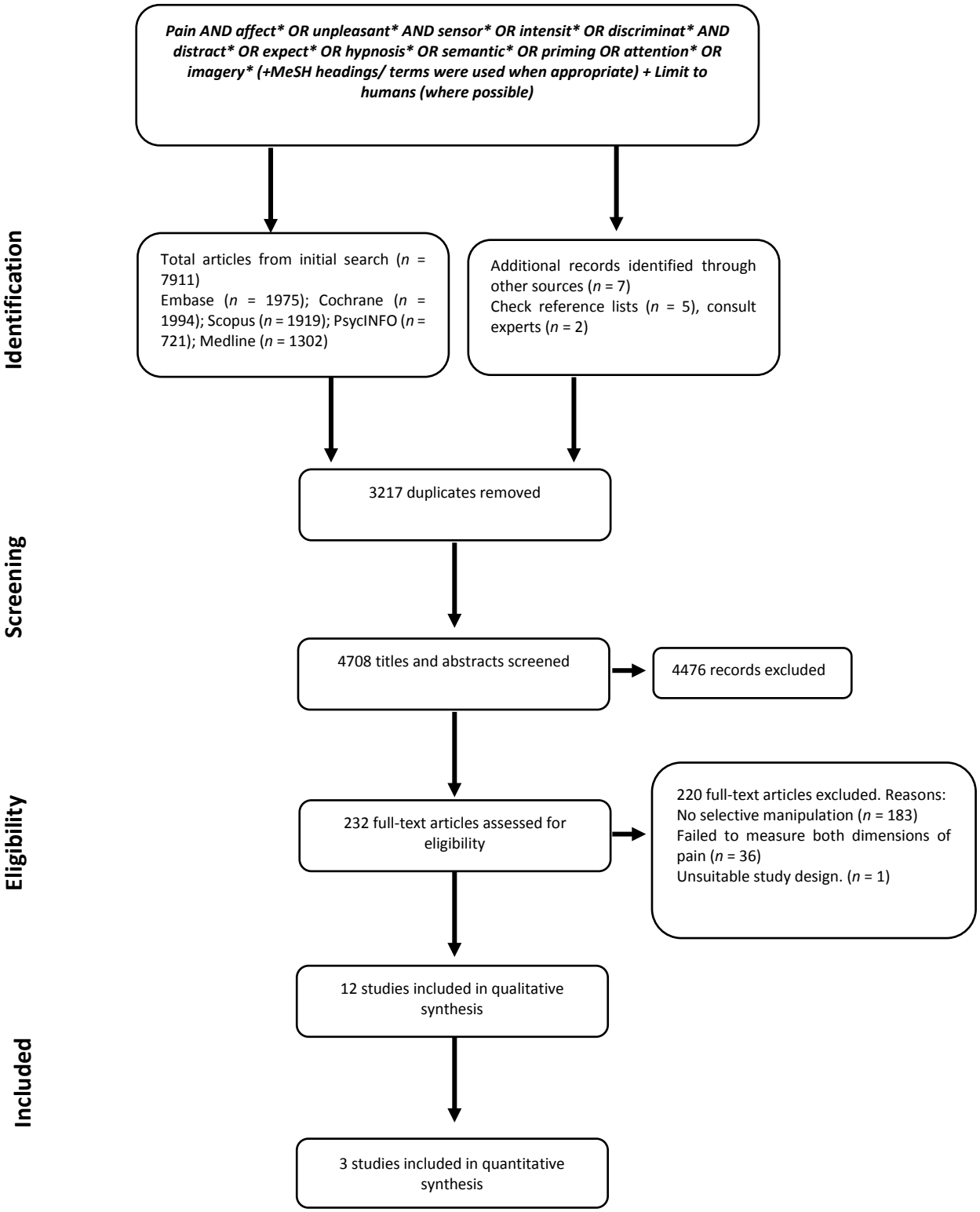
Appendices

Figure Caption

Figure 1. PRISMA flow chart of systematic search strategy.

Figure 2. The effectiveness of selective modulation of the sensory and affective dimensions of pain.

Figures


Pain AND affect* OR unpleasant* AND sensor* OR intensit* OR discriminat* AND distract* OR expect* OR hypnosis* OR semantic* OR priming OR attention* OR imagery* (+MeSH headings/ terms were used when appropriate) + Limit to humans (where possible)
Identification
Total articles from initial search (n = 7911)
Embase (n = 1975); Cochrane (n = 1994); Scopus (n = 1919); PsycINFO (n = 721); Medline (n = 1302)
Additional records identified through other sources (n = 7)
Check reference lists (n = 5), consult experts (n = 2)
3217 duplicates removed
Screening
4708 titles and abstracts screened
4476 records excluded
Eligibility
232 full-text articles assessed for eligibility
220 full-text articles excluded. Reasons:
No selective manipulation (n = 183)
Failed to measure both dimensions of pain (n = 36)
Unsuitable study design. (n = 1)
Included
12 studies included in qualitative synthesis
3 studies included in quantitative synthesis

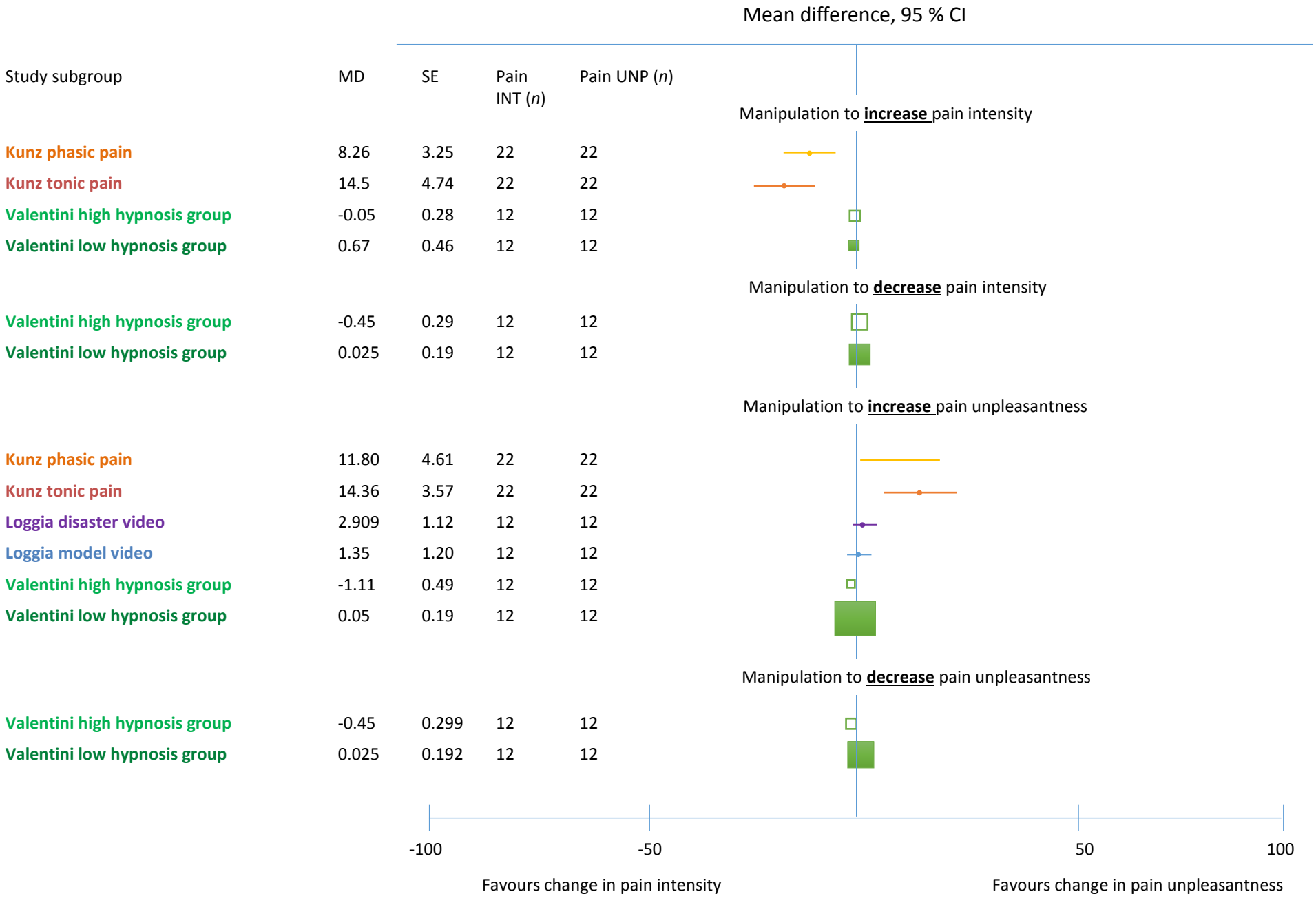

Mean difference, 95 % CI
Study subgroup
MD
SE
Pain INT (n)
Pain UNP (n)
Manipulation to increase pain intensity
Kunz phasic pain 8.26 3.25 22 22
Kunz tonic pain 14.5 4.74 22 22
Valentini high hypnosis group -0.05 0.28 12 12
Valentini low hypnosis group 0.67 0.46 12 12
Manipulation to decrease pain intensity
Valentini high hypnosis group -0.45 0.29 12 12
Valentini low hypnosis group 0.025 0.19 12 12
Manipulation to increase pain unpleasantness
Kunz phasic pain 11.80 4.61 22 22
Kunz tonic pain 14.36 3.57 22 22
Loggia disaster video 2.909 1.12 12 12
Loggia model video 1.35 1.20 12 12
Valentini high hypnosis group -1.11 0.49 12 12
Valentini low hypnosis group 0.05 0.19 12 12
Manipulation to decrease pain unpleasantness
Valentini high hypnosis group -0.45 0.299 12 12
Valentini low hypnosis group 0.025 0.192 12 12
-100
-50
50
100
Favours change in pain intensity
Favours change in pain unpleasantness

Table 1. Key characteristics of selected articles

Author	Year	Sample size (*n*) [Females:Males]	Mean age (years)	Cognitive manipulation	Scale type	Sensory pain anchors (Pain intensity)	Affective pain anchors (Pain unpleasantness)
Hypnotic induction with suggestion							
Dahlgren et al.	1995	32 [17:15]	N/R**	Hypnotic suggestion for relaxation or analgesia	VRS	Sensory list of words	Affective list of words
Rainville et al.	1997	8 [3:5]	19-53**	Hypnotic suggestion to ↓ and ↑ pain UNP	NRS	0: No burning, pricking, stinging sensation 100: Extremely intense sensation	0: Not at all UNP 100: Extremely UNP
Rainville et al.	1999	**Exp 1**: 17 [5:12] **Exp 2**: 20 [7:13] **Exp 3**: 22 [10:12]	**Exp 1:** 22.4 ± 6.0 Controls: 20.0 ± 0.8 **Exp 2:** 27.2 ± 9.8 **Exp 3:** 24.3 ± 4.6	Exp 1: Hypnotic suggestion to ↓ pain UNP Exp 2: Hypnotic suggestion to ↓ & ↑ pain UNP Exp 3: Hypnotic suggestion to ↓ & ↑ pain INT	VAS	Burning, pricking, stinging sensation	N/R**
Hofbauer et al.	2001	10 [6:4]	24.2 20-35	Hypnotic suggestion to ↓ and ↑ pain INT	MES	N/R**	N/R**
Valentini et al.	2013	24 [24:0]	22.7 ± 2.3	Hypnotic suggestion to alter pain INT or UNP	VAS	0: No pricking/ burning/ itching 101: Intolerable intensity	0: Not at all UNP 101: Intolerable UNP
Meditation							
Perlman et al.	2010	19 [9:10]	Experimental: 48.1 ± 10.7 Controls: 47.1 ± 11.8	Meditation	NRS	N/R**	N/R**
Grant & Rainville	2009	28 [10:18]	Experimental: 33.7 ± 10.99 Controls: 34.38 ± 10.18	Meditation	VAS	0: Not painful 10: Extremely painful	0: Not UNP 10: Extremely UNP
Valence induction with exteroceptive stimuli							
Kenntner-Mabiala et al.	2007	54 [27:27]	18-40	Picture valence and attention	VAS	0: No pressure 100: Just noticeable pain 200: Unbearable pain	-100: Very UNP 100: Very PL

Kenntner-Mabiala et al.	2008	30 [15:15]	26.5 ± 5.2	Picture valence and attention	VAS**	0: No sensation 4: Just noticeable pain 10: Unbearable pain	0: Neutral 10: Very UNP
Loggia et al.	2008	12 [12:0]	51.3 ± 3.5	Video valence	NRS	0: No heat 200: Most intense pain tolerable	N/R**
Villemure et al.	2009	14 [9:5]	23.0 ± 3.0	Odour and attention	NRS	0: No pain 10: Most intense pain tolerable	0: Neutral 10: Extremely UNP
Suggestion alone							
Kunz et al.	2012	22 [10:12]	22.7 ± 3.8	Suggestion to ↑ pain UNP or INT	VAS	0: No pain 100: Extremely strong pain	0: No pain 100: Extremely UNP pain

** Unclear in manuscript/ unable to obtain further information. N/R: Not reported. UNP: Pain unpleasantness. PL: Pleasant. INT: Pain intensity. VAS: Visual analogue scale.

NRS: Numeric rating scale. MES: Magnitude estimation scale. VRS: Verbal rating scale.

Table 2. Risk-of-bias assessment of included studies

Author (year)	Selection bias	Performance and detection bias	Cognitive technique	Measurement bias	Statistical methods	Reporting
Hypnotic induction with suggestion						
Dahlgren et al. (1995)	✖	?	✓	✖	✓	✖
Rainville et al. (1997)	✖	✖	✖	?	?	✖
Rainville et al. (1999)	✖	?	✖	✓	✖	✓
Hofbauer et al.(2001)	✖	✖	✖	✓	✖	✓
Valentini et al. (2013)	✖	✖	✖	✓	✓	✓
Meditation						
Grant & Rainville (2010)	✖	✖	✖	✓	✓	✓
Perlman et al. (2009)	✖	?	✖	✖	✖	✓
Valence induction with exteroceptive stimuli						
Kenntner-Mabiala et al. (2007)	✖	?	✓	✓	✖	✓
Kenntner-Mabiala et al (2008)	?	?	✓	✓	✖	✓
Loggia et al. (2008)	✖	?	✓	✓	✖	✓
Villemure et al. (2009)	✖	?	✓	✓	✓	✓
Suggestion alone						
Kunz et al. (2012)	✖	✖	✓	✓	✖	✓

X: High bias. ✔: Low bias. ?: Unclear (unclear in manuscript/ unable to obtain further information)

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

4 Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

4. Placing stimuli in order: Conflicts between the choices of researchers and participants.

The perceptual discrimination literature highlights the fact that differences between experimental stimuli are frequently manipulated and sequenced according to researchers' own perceptions rather than participants' perceptions. Failure to confirm whether researchers' and participants' perceptions are congruent could result in the incorrect interpretation of responses. Therefore, in order to investigate the extent of generalisation of a conditioned response, the order of the proposed experimental stimuli must first be established.

Chapter 4 tests whether respondents intuitively sequenced the eight experimental hand posture stimuli the same way as the researchers did.

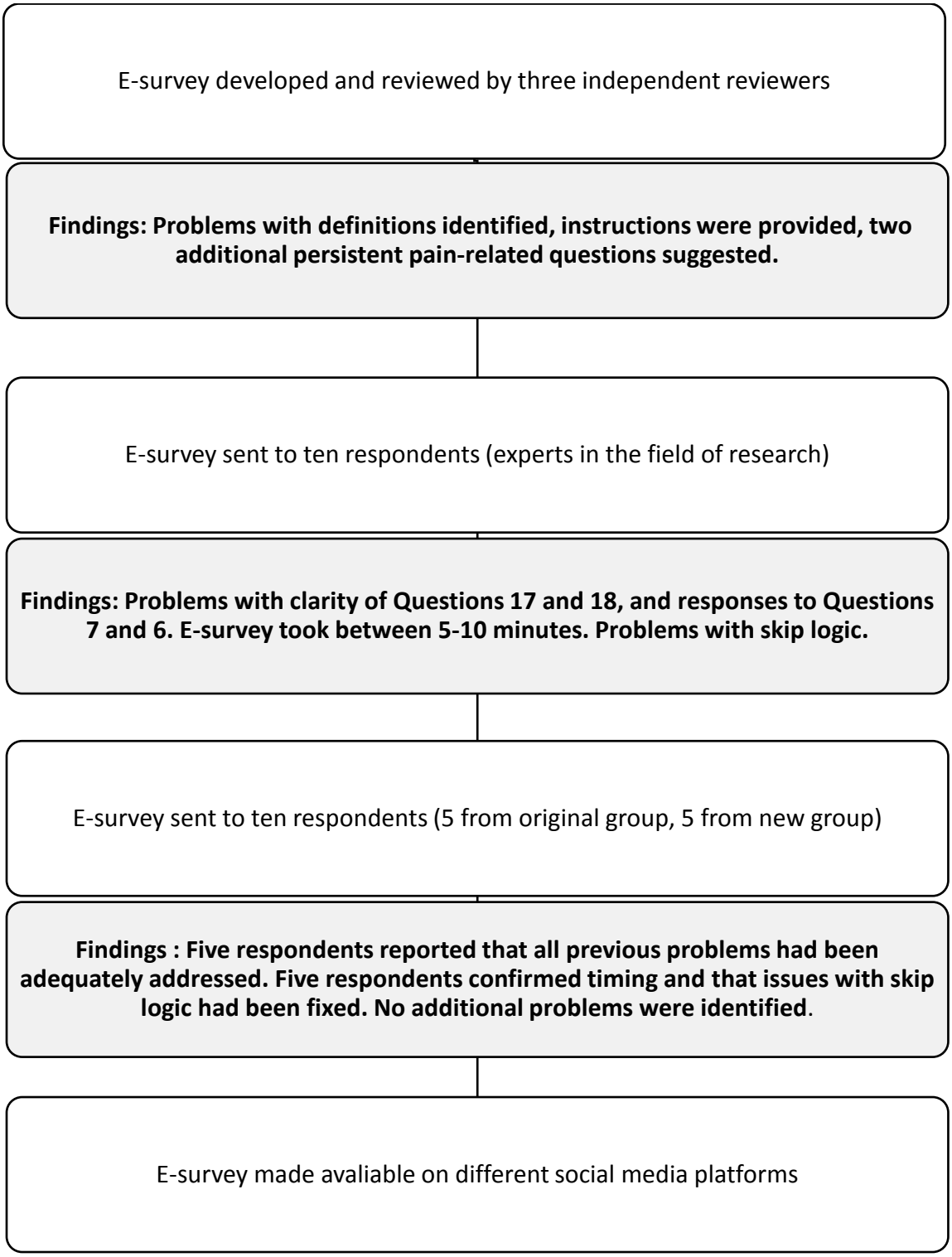


Figure 4.1. Piloting results for Chapter 4

Title: Placing stimuli in order: Conflicts between the choices of researchers and participants.

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Abstract

Background

Discriminating between stimuli that form a compound stimulus is an important process in perceptual learning. How a person discriminates between these stimuli has become a topic of interest in many different fields of research. Most recently, research within the associative learning field has brought to light the importance of assessing perceptual discrimination in studies of stimulus generalisation. However, while the concept of assessing perceptual discrimination has received a lot of attention, how the stimuli are arranged has received less attention. Therefore, this study investigated whether participants' intuitive arrangement of the proposed experimental hand posture stimuli was the same as the researchers' arrangement.

Methods

An e-survey was made available on different social media platforms. Respondents were asked a series of questions relating to their current and past pain experience, hand pain experience and demographics. The final two questions in this survey asked participants to arrange the proposed hand posture stimuli into a sequence and to explain their reasons for choosing that sequence.

Results

Of the 150 respondents who had completed the e-survey, 120 arranged the proposed experimental hand posture stimuli in the same way as the researchers. Differences in respondents' past and current pain experiences were not associated with differences in their arrangements of the hand posture stimuli.

Conclusion

The arrangement of the proposed experimental hand posture stimuli was confirmed and could reliably be used in stimulus generalisation studies. Most participants arranged the stimuli in the same as the researchers, confirming the validity of this stimulus sequence for studies of stimulus generalisation.

Introduction

Perceptual learning is an everyday occurrence, and discrimination between stimuli is a major part of the learning process (Goldstone, 1998). Improving the skill of perceptual learning often involves improving the ability to discriminate between two different stimuli (Pear, 2001). *Perceptual discrimination* is defined as the ability to identify that one stimulus is different from another stimulus in at least one dimension (Wiech, Ploner, & Tracey, 2008). This might range from the ability to discriminate between two different musical tones to the ability to discriminate between complex temporal or spatial stimuli (e.g. between a tumour and a bone on an x-ray; Goldstone, 1998). Recent acknowledgement that perceptual discrimination plays an important role in multiple processes has gained traction. This has prompted multiple studies from a range of fields to identify and investigate the role that perceptual discrimination plays in the maladaptation of different cognitive and perceptual processes (Goldstone, 1998; Jamshidian, Hubbard, & Jewell, 2014; Struyf, Zaman, Vervliet, & Van Diest, 2015; Vervliet & Van, 2015; Wiech et al., 2008; Zaman, De Peuter, Van Diest, Van den Bergh, & Vlaeyen, 2016; Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015). Of particular interest is the research that has identified that perceptual discrimination plays a role in the experience of persistent pain (Vervliet & Van, 2015). Some studies in persistent pain conditions have investigated the extent to which an individual can discriminate between different touch stimuli (Flor, Denke, Schaefer, & Grüsser, 2001; Moseley & Wiech, 2009; Moseley, Zalucki, & Wiech, 2008; Peters & Schmidt, 1992; Pleger et al., 2006; Pleger et al., 2005). Other studies have used a classical conditioning paradigm to investigate whether people with persistent pain conditions can discriminate between different threatening and non-threatening stimuli, or between different non-painful stimuli.

This widespread interest in perceptual discrimination demands that experimental designs allow for its assessment (Struyf et al., 2015; Vervliet & Van, 2015). This can be difficult in studies that require participants to discriminate between exteroceptive stimuli such as images of human faces or human body parts. Such stimuli may be substituted for traditional geometric shapes or auditory cues to improve ecological validity. This is particularly common in classical conditioning studies. A further difficulty of generalisation in classical conditioning studies is that there is a need to investigate the generalisation of responses across a spectrum of stimuli that are arranged in order of similarity (i.e. a stimulus sequence; Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015). The concept of assessing the

perceptual discrimination of stimuli has received a lot of attention (Struyf et al., 2015). However, the concept of *how* the stimuli are arranged has been investigated less. The way in which stimuli are arranged in order of similarity is often based on researchers' own strategies and perceptions. While this may not be a problem if the stimuli used are simple and uni-dimensional (e.g. circles or squares), it may be problematic if the stimuli are more complex (multidimensional), such as faces or hands, because there are many dimensions that could be used to inform the arrangement of the stimuli (Goldstone, 1994, 1998).

The importance of how researchers generate a stimulus sequence should not be ignored, for two main reasons. Firstly, research shows that individuals' perceptions of how to discriminate between stimuli can be manipulated. For example, a study showed that by pre-exposing participants to stimuli before an experiment, they were better able to discriminate between those stimuli in the test phase, compared to those who had not been pre-exposed (Lavis & Mitchell, 2006). Secondly, the way individuals categorise stimuli is malleable (Li, Howard, Parrish, & Gottfried, 2008; Morrongiello, 1992; Watanabe & Shimojo, 2005). For example, studies that trained participants to categorise stimuli based on a particular stimulus feature modulated the participants' perception of categorisation, so that participants discriminated between the stimuli based on the training, even if the training was counterintuitive to how the participant would have initially categorised the stimuli (Goldstone, 1994, 1998).

In the current study, we consider the *sequence* and the *strategy* used to sequence a set of stimuli as two separate entities. This distinction was deemed important, because an individual could use a different *strategy* than the researchers to sequence a set of stimuli, but still come up with the same *sequence.* Conversely, they could employ the same *strategy* as the researchers, yet end up with a different *sequence*. If a participant's sequence fails to be the same as the researchers', the implication is that the responses would be analysed according to the researchers' sequence. Therefore, the resultant analyses, stimulus categorisation and plotting of responses to the perceived similarities between different stimuli would be done according to the researchers' perception, and would not account for the possibility of a different sequence.

Methods

Aims

The primary aim of this electronic survey was to investigate whether the strategy that we used to sequence eight hand images was the same as that used by respondents. To reduce bias, respondents were not made aware that the sequence they selected would be used to confirm or refute our own sequence. The primary aim was investigated in two ways: in Question 17 of the survey, respondents were asked to provide a sequence. In Question 18, respondents were asked to provide their reasoning for choosing that sequence. Additionally, given the evidence of the malleability of perceptual discrimination, the secondary aim of this survey was to investigate whether chronic pain experience, current pain and different pain locations were associated with certain patterns of sequencing. For example, an individual with hand pain may arrange images of hand positions into a sequence that reflects their experience of the most painful hand position to the least painful. Therefore, the respondents' sequences from each group (groups same and different) were analysed to determine whether there were distinct patterns of sequences within each group.

Researchers' proposed sequence template

Figure 1 illustrates the proposed sequence of eight hand images, referred to as the sequence template. The strategy used by the authors to sequence the hand images reflected a gradual movement of hand positions, from a closed fist to an open hand (Figure 1). It is this sequence and strategy that was compared against those of the respondents. The two main reasons for the chosen sequence were: 1) this sequence and strategy had been used in a previous experimental study (Meulders, Harvie, Moseley, & Vlaeyen, 2015) and 2) it was deemed that the changes in the hand position were the most notable dimensional difference between each hand image.

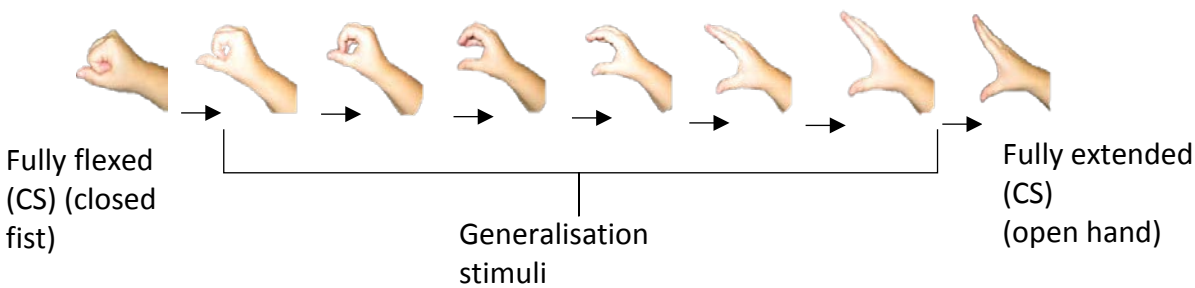


Figure 1. Sequence template.

General outline of the survey

An electronic survey was developed to evaluate respondents' sequencing of specific hand images. The survey was developed by the primary investigator (KAT) and was hosted by SurveyMonkey (www.surveymonkey.com). The Checklist for Reporting Results of Internet E-Surveys (CHERRIES) was used to guide reporting of this survey's methods and results (Eysenbach, 2004).

Piloting

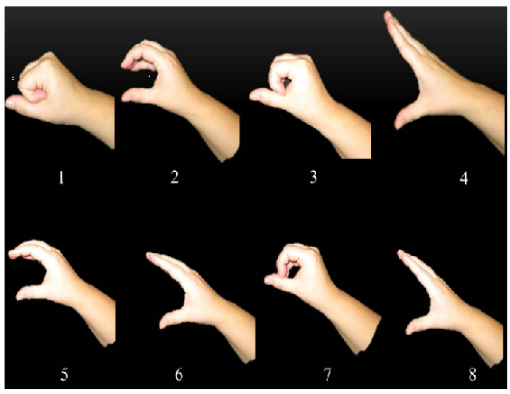
The initial survey was developed by KAT and independently reviewed by three people (SJ, VJM & GLM) to confirm the clarity and comprehensibility of the questions. Following feedback from these reviewers, the survey was edited and piloted using 15 volunteers. Volunteers completed the survey and provided feedback on its structure and on the duration and phrasing of the questions. Further refinement and clarification was performed on the basis of this feedback. During the piloting process, volunteers took between five and ten minutes to complete the survey, and reported that this was acceptable. Ethical approval to conduct this online survey was provided by the Human Research Ethics Committee of the University of South Australia (protocol number: 0000034504).

Survey outline

The survey comprised 18 questions in a standardised order, covering demographics, pain experience, arthritis and hand injury information. Certain questions were linked, so that if participants answered 'no' to an initial question (e.g. 'Do you have arthritis in your hands?') then follow-up questions (e.g. 'What type of arthritis have you been diagnosed with in your hands?') would be skipped. The final two questions related to respondents' sequencing of the hand images and the reason for the chosen sequence (Figure 2). The full survey is outlined in Attachment A.

Question 17

Please write your sequence here (please check that you have EIGHT numbers and no repetitions).



Question 18

We are interested in the way you have ordered the images. In the text box below please explain your reasoning for the sequence you have chosen.

Figure 2. Hand image questions.

The survey was distributed via a range of social media portals (Facebook, Twitter and the Body in Mind website) and word-of-mouth. Participation was voluntary and no remuneration or incentive was given for completing the survey. Individuals 18 years of age or older, who had access to a computer and the internet, were eligible to participate. The first two pages outlined survey information, confidentiality, and a brief explanation of how to access the results. The respondent was required to choose the 'I agree' response on both pages in order to continue with the survey. If consent was not provided in this manner, the survey ended immediately. Access to the survey was restricted to one response per internet protocol (IP) address to minimise the potential for a single respondent to complete the survey multiple times. Computer IP address details were not collected during data collection, to ensure anonymity.

Data processing

Responses missing data for Questions 17 and 18 (relating to the main outcome) were removed. The following processes were undertaken to clean up the data: (1) responses

entered as 'other' and then specified in free text were manually coded; (2) occupations were categorised using The Australian and New Zealand Standard Classification of Occupations (ANZSCO; Attachment B; Trewin, Trewin, & Pink, 2006), and responses 'retired, 'homemaker', 'fulltime student', and 'unable to work' were categorised as 'not in the workforce'. (3) For Question eight ('*Where in your body did you or do you feel pain?*'), the pain locations were grouped as 'upper body' (above the hips), 'lower body' (below the hips) or 'whole body' (above and below the hips). However, if the respondents specified hand/s as a pain location, then this was grouped as "hands". (4) Responses to Question 17, which asked respondents to sequence the hand images, were classified as *Group S (i.e. Group Same)* or *Group D (i.e. Group Different)*, according to whether the chosen sequence was the same as, or different to, the sequence template.

Results

The survey link was made available on July 1st 2015 through social media and was accessible for a total of ten weeks. The survey was accessed 185 times. Six respondents did not provide consent. Of the remaining 179 consenting respondents, 29 failed to complete the survey, so their data was discarded. Of the 150 complete surveys suitable for analysis, the majority (*n* = 119) occurred in July.

The results are presented in three ways: 1) results from the full sample (Table 1), 2) the between-group (*Group S* versus *Group D*) results (Table 2) and, 3) and according to whether reported chronic pain experiences, current pain and pain locations were associated with certain patterns of sequencing.

Results from the full sample

The majority of respondents were female (*n* = 116, 77%), with the 30-39 year age range most highly represented (*n* = 39, 26%). 'Professionals' was the highest represented occupational grouping (*n* = 85, 57%). More than half of all respondents (*n* = 92, 61%) reported experiencing pain for three months or longer. Just over half of all respondents (*n* = 77, 51%) reported current pain (at the time of completing the survey). A smaller number of respondents (*n* = 32, 21%) reported that they had arthritis in their hands, with osteoarthritis

being the most commonly reported type (*n* = 17, 53%). A large proportion of respondents (*n* = 70, 47%) reported having injured their hands, with 29% (*n* = 20 of 70 respondents) having injured only the left hand, and 31% (*n* = 22 of 70) having injured only the right hand, and 40% (*n* = 28 of 70 respondents) having injured both hands. Of those respondents who reported having injured their hand/s, 60% (*n* = 42 of 70 respondents) reported still experiencing pain.

Table 1. Results from the full sample

Demographics		***n***	**%**
Sample	**Total**	**150**	
	Male	34	(23)
	Female	116	(77)
Age range (years)	18-20	3	(2)
	21-29	28	(19)
	30-39	39	(26)
	40-49	27	(18)
	50-59	36	(24)
	60<	17	(11)
Occupation	Professionals	85	(57)
	Managers	2	(1)
	Associate Professionals	27	(18)
	Intermediate Clerical, sales & Service	18	(12)
	Workers	2	(1)
	Not in workforce	16	(11)
Pain experience			
Experienced pain for 3 months	Yes	92	(61)
	No	58	(39)
Currently have pain	Yes	77	(51)
	No	73	(49)
Injury and disability			
Arthritis in hand	Total	**32**	**(21)**
	Osteoarthritis	17	(53)
	Rheumatoid	3	(9)
	Psoriatic	1	(3)
	Non-responders	11	(34)
Previous hand injury	**Total**	**70**	**(47)**
	Left hand	20	(29)
	Right hand	22	(31)
	Both hands	28	(40)
Hand pain	Still painful	42	(60)
	Not painful	28	(40)
Pain in uninjured hand	Pain in uninjured hand	18	(26)
	No pain in uninjured hand	52	(74)

Results from Group S versus Group D

Eighty percent of respondents' (120 of 150) chosen sequences were the same as the sequence template (i.e. Group S; Table 2). Twenty percent of respondents' (30 of 150) chosen sequences were different from the sequence template (i.e. Group D). There was little difference in gender or occupation between the two groups. There was a slight difference in age ranges, with the 30-39-year category being most common in Group S (31 of 120 respondents, 26%), while the 50-59-year category was most common in Group D (11 of 30 respondents, 37%). A higher percentage of respondents reported having experienced pain for longer than three months in Group D (27 of 30 respondents, 87%) compared to Group S (65 of 120 respondents, 53%). However, current pain was comparable between the two groups, with 49% of Group S (59 of 120 respondents) and 60% of Group D (18 of 30 respondents) reporting current pain. A larger number of respondents in Group D reported having arthritis in their hands (12 of 30 respondents, 40%) with osteoarthritis being the most common type (11 of 12 respondents, 92%), compared to Group S. Fifty percent of respondents in Group D (15 of 30 respondents) reported having had an injured hand or hands. Of the respondents in Group D who reported having had an injured hand/s, 73% (11 of 15) of these respondents reported still experiencing pain in their hand/s.

Table 2. Results from *Group Same* versus *Group Different*

GROUP SAME		(n)	(%)	GROUP DIFFERENT	(n)	(%)
DEMOGRAPHICS						
Sample	Total	120	(80)	Total	30	(20)
	Female	92	(77)	Female	23	(77)
	Male	28	(23)	Male	7	(23)
Age Range (years)	18-20	2	(1)	18-20	1	(3)
	21-29	25	(21)	21-29	3	(10)
	30-39*	**31**	**(26)**	30-39	8	(27)
	40-49	26	(22)	40-49	1	(3)
	50-59	25	(21)	**50-59***	**11**	**(37)**
	60<	11	(9)	60<	6	(20)
Occupation	Professionals	69	(57)	Professionals	16	(53)
	Managers	1	(1)	Managers	1	(3)
	Associate Professionals	22	(18)	Associate Professionals	5	(16)
	Intermediate Clerical, sales & Service	16	(14)	Intermediate Clerical, sales & service	2	(6)
	Workers	1	(1)	Workers	1	(3)
	Not in workforce	11	(9)	Not in workforce	5	(16)
PAIN EXPERIENCE						
Experienced pain for >3 months	**Yes***	**65**	**(53)**	**Yes***	**27**	**(87)**
	No	54	(47)	No	4	(13)
Currently have pain	Yes	59	(49)	Yes	18	(60)
	No	61	(51)	No	12	(40)
INJURY AND DISABILITY						
Arthritis in hands	**Total****	**20**	**(17)**	**Total****	**12**	**(40)**
(*Only 9 respondents for	**Osteoarthritis****	6	(30)	**Osteoarthritis****	11	(92)
arthritis type)	Rheumatoid	2	(10)	Rheumatoid	1	(8)
	Psoriatic	1	(5)	Psoriatic	0	(0)
	Non-responders	11	(55)			
Previous hand injury	Total	55	(46)	Total	15	(50)
	Left hand	15	(27)	Left hand	5	(33)
	Right hand**	**20**	**(36)**	**Right hand****	**2**	**(13)**

	Both hands	20	(36)	Both hands	8	(54)
Hand pain	**Still painful***	**31**	**(56)**	**Still painful***	**11**	**(73)**
	Not painful	24	(44)	Not painful	4	(27)
Pain in uninjured hand	**Pain in uninjured hand***	**16**	**(29)**	**Pain in uninjured hand***	**2**	**(13)**
	No pain in uninjured hand*	**39**	**(71)**	**No pain in uninjured hand***	**13**	**(87)**

*: a difference of 10% or more between the two groups for that variable, **: a difference of 20% or more between the two groups for that variable

Results according to chosen sequence (Question 17)

The results for Question 17 yielded two main groups: Group Same (S) selected a sequence that was the same as the sequence template, and Group Different (D) selected a different sequence. Further analysis of results from Group D identified two distinct subgroups (Figure 3). The first subgroup had only one difference in each of their sequence responses. That is, only one of the eight hand images were sequenced differently, compared to the sequence template. The second subgroup had six or more differences in their sequences.

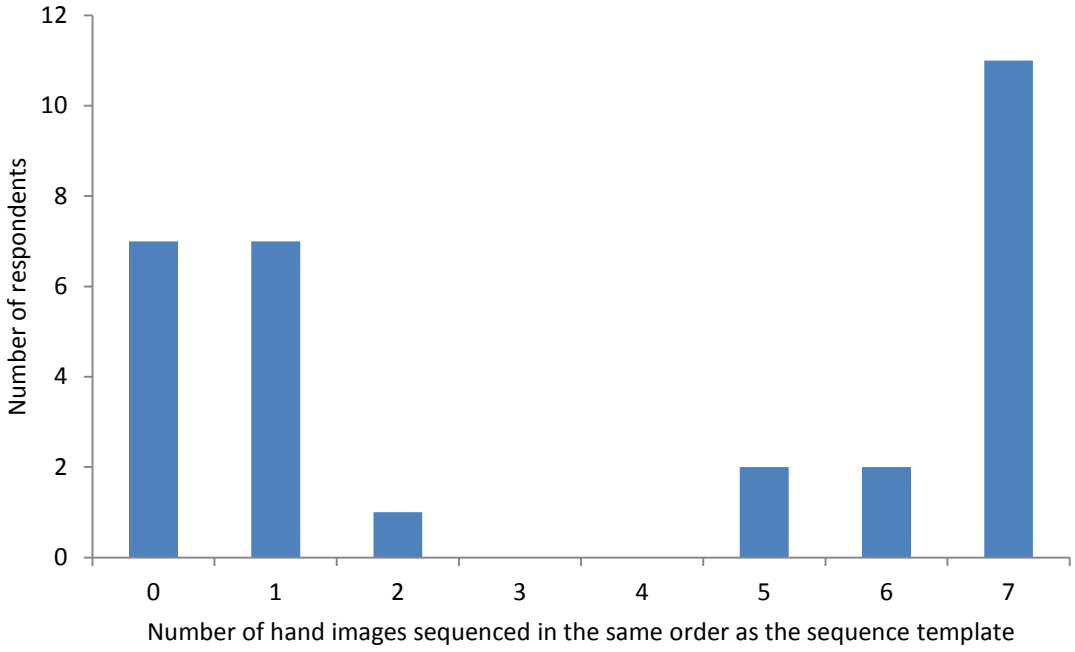


Figure 3. Number of hand images in Group D that were the same as the sequence template.

Strategy for chosen sequence (Question 18)

The majority of the respondents (144 of 150, 96%) reported that the strategy they used to sequence the hand images reflected a gradual progression of hand positions from a closed fist to an open hand. Within Group D, a small percentage of respondents used different sequencing strategies. There was a small percentage of respondents (24 of 144, 17%) who reported using the same strategy as the researchers (gradual movement from closed fist to open hand) but whose sequence (which was provided in the text box for Question 17), was different. There appeared to be two main reasons for this. One, some respondents (8 of 24, 33%) made an error (they forgot a number and repeated another number, e.g. 8, 7, **4**, 5, 3, 2, 6, **4**) and two, some respondents (16 of 24, 67%) swapped two similar hand images so that the sequence was no longer the same as the template (for an example, see Figure 4). There were a small number of respondents whose sequencing strategies were different, yet they

did not have any other similar characteristics (e.g. pain experience or age). A couple of respondents (2 of 3, 70%) reported that their chosen sequence reflected the numbers that the hand images were assigned (e.g. 1, 2, 3, 4, 5, 6, 7, 8). One (of 3, 30 %) reported that their chosen sequence reflected the action of grabbing and then releasing an object (for an example, see Figure 5). The other three respondents whose strategies differed from the researchers' strategy all reported having hand pain and were analysed further, as per the section below.



Figure 4. Hand images that were most commonly switched around.



Figure 5. Example of a different sequence of hand images – grabbing and releasing an object.

Investigation into whether chronic pain experience, current pain and pain location are associated with certain patterns of sequencing.

Respondents were allocated to groups depending on their responses to the chronic pain experience, current pain and pain location questions. Respondents who reported experiencing pain for three months or longer were grouped together, and their patterns of hand image sequencing were analysed. Respondents who reported pain at the time of the survey, and respondents who had reported a pain location were also grouped and analysed. Within the total chronic pain experience group (90 of 150 respondents), the majority (64 of 90, 71%) sequenced the hand images the same as the sequence template. Similarly, within the current pain group (77 of 150 respondents, 51%) the majority (59 of 77, 77%) selected the same sequence as the template. However, for pain location respondents, a small percentage (3 of 77, 4%) reported that their chosen sequence reflected their experience of the least painful hand position to the most painful hand position (for an example, see Figure 6). All three of these respondents had hand pain.

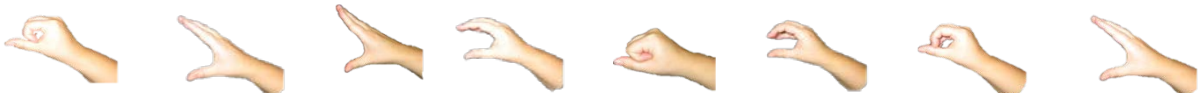


Figure 6. Example of a different sequence of hand images - least painful hand position to the most painful

Discussion

This survey investigated whether the strategy that we (the researchers) used to sequence eight hand images was the same as the strategy that respondents used to sequence the same eight hand images. This aim was investigated in two ways: first, the sequence provided by the respondents (Question 17) was examined. The results demonstrated that the majority (80%) of respondents' sequences were the same as the sequence template (i.e. the researchers' sequence). Nearly half (43%) of the remaining 20% of respondents whose sequences differed had only one difference in their sequence. These results indicate that the sequence template (Figure 1) reflected most respondents' preferred sequencing of the hand images. Second, the strategy the respondents used to sequence the hand images (Question 18) was examined. The majority (96%) of respondents' strategies were similar to the researchers'. That is, the sequencing of the hand images reflected a gradual movement of the hand from a closed fist to an open hand (Figure 1).

This study also investigated whether there were demographic, arthritis and pain experience differences between the respondents whose sequences were the same as the template (Group S) and those whose sequences differed (Group D). The main demographic difference between Groups S and D was in age: the majority of Group S were younger (30-39 years) than the majority of Group D (50-59 years). In this survey, age appeared to be associated with differences in sequencing. This was not the case for gender or occupation. Certainly, age-related changes in cognitive processes have been extensively investigated (Prull, Gabrieli, & Bunge, 2000; Rypma, Berger, Genova, Rebbechi, & D'Esposito, 2005; West, 1996). One example is a study by Rypma et al. (2005) which examined age-related changes in cortical blood flow during perception and memory. This study demonstrated an age-related functional decline that affected cognitive processes. There were fewer respondents who had arthritis in Group S (17%) than in Group D (40%). However, Group S had a greater percentage of respondents in the younger age range than Group D. This difference was expected, as the prevalence of arthritis increases with age (Reginster, 2002). The differences between the two groups in terms of age and arthritis incidence appeared to be associated with differences in sequencing of the hand images.

Although this study did not test for a directional or modulatory influence, other studies have shown that many variables, such as previous experience, arousal and emotional association, can alter perceptions of stimuli (Vervliet & Van, 2015). Additionally, previous training or exposure has been shown to alter the categorisation of stimuli (Goldstone, 1994). In this study, the two sequence groups (S & D) differed in their chronic pain histories: 87% of Group D reported having experienced chronic pain, compared with only 53% of Group S. This suggests that chronic pain experience may be associated with certain sequencing preferences. This finding was not surprising, because the literature reports that participants' pain history influences their perception of which stimuli most resemble each other (Eads, Moseley, & Hillier, 2015; Moseley, 2008; Moseley et al., 2008).

Interestingly, there were more respondents in Group S who reported right hand pain and pain in their uninjured hand than in Group D. This finding is of interest, as we expected that the presence of hand pain may have altered the sequencing of the hand images. For example, respondents with hand pain may have sequenced them according to the most to least painful hand position. A study conducted by Pincus and Morley (2001) and colleagues showed that patients with chronic pain demonstrated selective processing of pain and illness-related stimuli. Therefore, we expected more respondents who had hand pain to be in Group D. A caveat, at this point, is that the numbers of respondents who had hand pain were small in both groups. Furthermore, analysis of the strategy used to sequence the hand images did find a small number of respondents who sequenced the hand images according to their pain experience. These conflicting findings suggest that the minor observations made in this survey should be treated with caution. However, the finding that a small number of respondents used different strategies should not be entirely ignored. This is because these differences highlight a potential interaction of variables that determine how stimuli are sequenced (Zaman et al., 2016; See Results: *Investigation into whether chronic pain experience, current pain and pain location, is associated with certain patterns of sequencing)*.

Even when the respondents were regrouped according to chronic pain experience, current hand pain or pain location, most respondents still sequenced the hand images the same as the researchers. This led us to ask, 'Why was the strategy of sequencing the hand images from a closed fist to an open hand so prevalent?' We propose two possible explanations.

First, the strategy used to sequence the hand images in this particular sequence may represent an intuitive motor movement or pattern (Zaman et al., 2016). That is, moving the hand from a closed fist to an open hand may merely represent a familiar movement sequence. Second, a large proportion of respondents in both groups were either health professionals or working in health-related occupations. Certainly, studies have shown that training influences how participants categorise stimuli (Adler et al., 2005; Goldstone, 1994, 1998). Therefore, the strategy used for sequencing the hand images may reflect the anatomical, movement-based training that health-related professionals receive.

This survey highlighted two experimental components for future studies to consider, particularly conditioning and generalisation studies. First, it is important that experimental stimuli are chosen to closely represent the real-life stimuli that evoke the same responses as those that the experiment aims to evoke (Hamm, Vaitl, & Lang, 1989; Till, Stanley, & Priluck, 2008). In classical conditioning studies, employing more naturalistic conditioning stimuli has been shown to enhance the conditioned response and increase resistance to extinction (Domjan, 2005). Similarly, other studies by Öhman and Mineka (2001, 2003), have found stronger fear conditioning when the CS was an image of snakes rather than an image of mushrooms or flowers. Furthermore, when exploring phobias, the experimental stimulus is usually representative of the phobia (Davey, 1992). For example, the experimental stimuli chosen when investigating arachnophobia, are commonly spiders or pictures of spiders (Öhman & Mineka, 2001). This is so that the response evoked (fear of spiders) would be representative of the presumed initial unconditioned response (Öhman & Mineka, 2001). In pain research, experimental observations could be improved by using experimental stimuli that better represent the actual stimuli involved in real-life development of maladaptive processes (Hamm et al., 1989; Lissek et al., 2008; Till et al., 2008).

Second, studies need to consider the complex interaction between perception and other cognitive processes (Chubb, 2005). However, this is particularly important in classical conditioning studies of generalisation, where responses across a spectrum of stimuli are arranged in order of similarity. Failure to account for differences in participants' ability to discriminate between experimental stimuli, and for differences in how the stimuli would be arranged, can lead to researchers making false assumptions and conclusions (Struyf et al., 2015; Vervliet & Van, 2015). If, in the case of generalisation studies, the researcher has failed

to measure perceptual discrimination, the results can have two different explanations (Vervliet & Van, 2015). One is that the participant distinguishes between the CS and the GS, and because the GS resembles the CS, the meaning associated with the CS is extrapolated to the GS (Vervliet & Van, 2015), resulting in generalisation of the response because it is deemed appropriate. The second possible explanation is that the participant fails to discriminate between the CS and GS, but perceives the two stimuli as identical. In this case, the observation of the same response to the CS and its most similar GS cannot be called a 'generalised response', because the participant has responded to what they perceived to be two identical presentations of the CS (Vervliet & Van, 2015). The former explanation demonstrates stimulus generalisation, and the latter explanation demonstrates differences in participants' perceptual acuity. Research participants' spontaneous sequencing of stimuli should be assessed in experimental studies (Vervliet & Van, 2015). That is, if the researchers' strategy used to the sequence the stimuli is not the same as the participant's strategy, then what the researcher has perceived as the GS that resembles the CS the most could, in fact, be perceived by the participant as the GS that resembles the CS the least. While this is less of a problem when using stimuli that are only different from each other in one dimension (e.g. size or colour), it is certainly more of a problem when using stimuli that are multidimensional. This is because there are more dimensions that could be used to inform a sequence. Moreover, usually stimuli that are multidimensional tend to have higher ecological validity (e.g. human faces or hands). Use of ecologically-valid stimuli is particularly problematic regarding sequencing, because the meaning associated with higher ecological stimuli is unknown (Domjan, 2005). That is, we do not always know how one attributes meaning to a stimulus (even a seemingly de-contextualised visual stimulus), nor how that meaning may influence the way they sequence the stimuli (Domjan, 2005). Failure to assess this could result in an interpretation of the results that does not reflect the participant, who may have sequenced the stimuli based on a different meaning.

Four limitations within this study should be considered when interpreting the results. First, there was a large disparity in numbers between the two groups, which were created *post hoc*. Therefore, any differences between the two groups may reflect differences in group size rather than individual characteristics. Second, the limited range of respondent professions may mean that their sequencing was more representative of health-related professions than business or entertainment-type professions. Third, the potential error of

repeating the rating number by participants for Question 17 could have been eliminated by survey design. For example, the use of a drop-down box which allows participants to select the numbers and prevents the reuse of already selected numbers. Fourth, only a small number of respondents reported the presence of arthritis and hand pain. As a consequence, our ability to determine how these two variables affected the sequencing of the hand images is limited. A final and important point is that, while this survey found that the researchers' sequencing of the hand images was the same as that of the participants, it does not replace experimental manipulation checks.

Conclusion

This survey found that most respondents sequenced a set of hand images the same way as the researchers did. Therefore, this particular sequence could be used in experimental classical conditioning and stimulus generalisation studies, subject to a manipulation check. Furthermore, this survey suggests that future studies need to consider the use of ecologically-valid stimuli when investigating the perception and perceptual discrimination of experimental stimuli.

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Attachment A

Hand Postures and Pain

***1. Information: Hand images and Pain Survey**

The Body in Mind Research Group at the University of South Australia invites you to be part of an investigation of pain and learning. We are interested in the things that affect how we experience pain. A better understanding of this will help us to make sense of acute and chronic pain, and therefore improve preventative treatment in the future. Participants over the age of 18 are invited to take part.

This is a research study and your participation is entirely voluntary. This survey will collect data regarding different hand images and general pain information. There are a total of 18 questions to answer and there are three mandatory questions that require a response. The mandatory information required relates to your consent to participate in this survey, to be contacted again and sent a second survey. All data collected from you will be stored securely. The data collected from you is automatically altered so that you cannot be identified from your responses.

However, if you consent to being contacted again we will require an email address. Your email address will be kept secure and confidential. After completion of the follow-up process all emails will be deleted.

Question 17: if you tick the box 'yes', you are consenting to giving the researchers your email address and completing the survey again. You will be sent the link to a second survey in approximately three weeks' time. After the follow-up phase you will not be contacted again. All de-identified information will be used only for research purposes and the email addresses you provide will not be shared and will be deleted on completion of the follow-up phase.

By ticking I agree below you are acknowledging that you have read and understand the above information

- [ ] I agree
- [ ] I disagree

*2. CONSENT FORM

This project has been approved by the University of South Australia's Human Research Ethics Committee. If you have any ethical concerns about the project or questions about your rights as a participant please contact the Executive Officer of this Committee at +61 8 8302 3118 or via email vicki.allen@unisa.edu.au.

Project title: Hand Images and Pain Survey

Primary contact details:

Kerwin Talbot Phone: +61 (08) 83021295 Email: Kerwin.Talbot@mymail.unisa.edu.au

Consent conditions:

• I have read the Participant Information question one and the nature and purpose of the research project has been explained to me. I understand and agree to take part.

• I understand the purpose of the survey and my involvement in it.

• I understand that I may withdraw from the survey at any point.

• I understand that while information gained during the survey may be published, I will not be identified and my personal results will remain confidential and de-identified.

• I understand that the data obtained during the survey will be coded and de-identified.

• I understand that the de-identified data will be used for research.

By ticking the 'I agree' box you are consenting that you are 18 years or older and you consent to the responses you provide being used in research.

- [ ] I agree
- [ ] I disagree

3. Are you male or female?

- ◯ Female
- ◯ Male

4. What is your age?

- ◯ 17 or younger
- ◯ 18-20
- ◯ 21-29
- ◯ 30-39
- ◯ 40-49
- ◯ 50-59
- ◯ 60 or older

Hand Postures and Pain

5. Which of the following best describes your current occupation?

- ○ Healthcare Support Occupations
- ◉ Computer and Mathematical Occupations
- ○ Life, Physical, and Social Science Occupations
- ○ Construction and Extraction Occupations
- ○ Farming, Fishing, and Forestry Occupations
- ○ Arts, Design, Entertainment, Sports, and Media Occupations
- ○ Architecture and Engineering Occupations
- ○ Building and Grounds Cleaning and Maintenance Occupations
- ○ Production Occupations
- ○ Installation, Maintenance, and Repair Occupations
- ○ Legal Occupations
- ○ Management Occupations
- ○ Office and Administrative Support Occupations
- ○ Business and Financial Operations Occupations
- ○ Food Preparation and Serving Related Occupations
- ○ Personal Care and Service Occupations
- ○ Education, Training, and Library Occupations
- ○ Healthcare Practitioners and Technical Occupations
- ○ Sales and Related Occupations
- ○ Protective Service Occupations
- ○ Community and Social Service Occupations
- ○ Transportation and Materials Moving Occupations
- ○ Other (please specify)

6. Have you ever experienced pain for 3 months or longer?

- [ ] Yes
- [ ] No

7. Do you currently have pain?

- [ ] Yes
- [ ] No

***8. Where in your body did you or do you feel the pain?**

- [ ] Hand
- [ ] Forearm
- [ ] Upper arm
- [ ] Other (please specify)

9. Do you have arthritis in your hands?

- [ ] Yes
- [ ] No

10. Has your arthritis in your hands been diagnosed by a medical practitioner?

- [ ] Yes
- [ ] No

Other (please specify)

11. What type of arthritis have you been diagnosed with in your hands?

- ◯ Osteoarthritis
- ◯ Rheumatoid arthritis
- ◯ Psoriatic arthritis
- ◯ Ankylosing spondylitis

Other (please specify)

12. Have you ever injured your hand/s?

- [ ] Yes (only the left hand)
- [ ] Yes (both hands)
- [ ] Yes (only the right hand)
- [ ] No

Other (please specify)

Hand Postures and Pain

13. Do you experince pain in the injured hand/s?

- [ ] Yes
- [ ] No
- [ ] N/A

14. How often do you experience pain in the hand/s that was injured?

- [ ] Constantly
- [ ] Occasionally
- [ ] Seldom
- [ ] Never
- [ ] N/A

Other (please specify)

15. Do you ever experience pain in the hand that has never been injured?

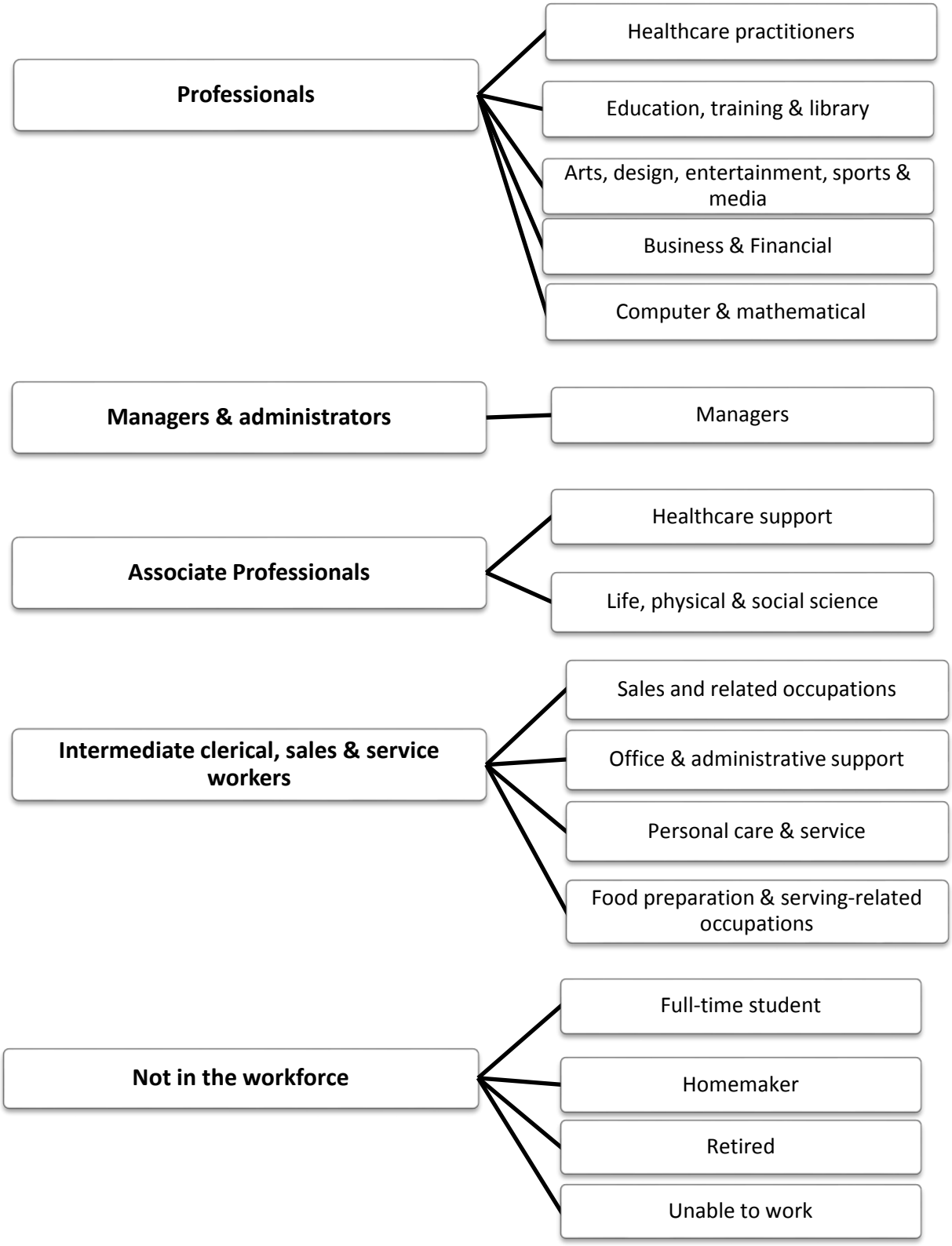
- [ ] Yes
- [ ] No
- [ ] N/A

16. How often do you experience pain in the hand that has never been injured?

- [ ] Constantly
- [ ] Occasionally
- [ ] Seldom
- [ ] Never
- [ ] N/A

Other (please specify)

Attachment B: Classification of occupations into major categories



Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

5 Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

5. Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

The imprecision hypothesis of chronic pain suggests that the more imprecise the encoding and subsequent cortical representation of a stimulus that causes a pain response, the more likely other, similar stimuli will resemble the original stimulus. Theoretically, the extent to which the pain response generalises to the similar stimuli is determined by how imprecise the original encoding and cortical representation was. The more imprecisely a painful event is represented cortically, the more likely it is that other stimuli that can elicit a pain response. Associative learning studies have shown both conditioning-induced hyperalgesia and generalisation of pain-related fear. However, whether conditioning-induced hyperalgesia generalises to novel but similar stimuli in healthy humans is yet to be determined.

Chapter 4 confirmed that the majority of respondents' intuitive sequencing of experimental hand posture stimuli was the same as the researchers'. Therefore, these hand posture stimuli are used as conditioned and generalisation stimuli for the study contained in this chapter.

This chapter tests the extent to which conditioning-induced hyperalgesia generalises to different hand posture stimuli in healthy adults. In order to test this hypothesis, the researchers first needed to investigate whether hyperalgesia could be a classically-conditioned response to hand posture stimuli, and then investigate the extent to which the conditioned response generalised to similar hand posture stimuli.

A laser stimulus was tested on 3 people, with variation of its spot size and pulse duration

Findings: A 4 mm spot size and 5 ms pulse duration provided the optimal laser stimulus

Trial of the proposed number and increment increase of the US on 3 participants to determine the consistency of the target ratings

Findings: 3 repeated stimulations with an incremental increase of 0.25 J was optimal

Trial of the pre-programmed coordinates and time taken for the robotic arm to move the laser hand piece to the next stimulation site.

Findings:

Distance (mm)	*Time taken (s)*	*Overlap of stimulation site*
5	10	Yes
8	**15**	**No**
10	20	No

Trial of the robotic arm movement pattern and mapped-out area on the dorsum of the hand

Findings: An up-across-down pattern, and a mapped-out area of 70 x 70mm, were chosen

Entire experiment programmed and trialled on 5 participants.

Findings

I. **3000 ms was sufficient time to rate the US expectancy**
II. **The questions displayed on the screen were identified as being clear**
III. **The participant instructions needed to be changed due to problems with the explanation provided for the rating of US expectancy**
IV. **The time allocated between trials was 10 seconds**
V. **The time taken to complete the entire experiment was 60 minutes**

Entire experiment programmed and trialled on 5 participants

Findings:

VI. **The participant instructions were considered to be clear.**

Piloting completed. Recruitment for the study began.

Figure 5.1. Piloting results for Chapter

Title: Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

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Abstract

Classical conditioning studies have demonstrated how a neutral conditioned stimulus previously associated with a highly painful unconditioned stimulus can elicit an amplified pain response when paired with a less painful stimulus (i.e. conditioning-induced hyperalgesia). However, few studies have investigated whether conditioning-induced hyperalgesia generalises to similar but novel stimuli. The current study tests whether a conditioned response of hyperalgesia decreases as the similarity of generalisation stimuli (hand posture images) to a CS+ (a highly painful hand posture image) decreases in healthy adult humans. We used a differential trace classical conditioning paradigm in which one hand posture conditioned stimulus was paired with a highly painful laser stimulus (CS+), while another hand posture conditioned stimulus was unpaired (CS-). Following acquisition, a previously established, moderately painful stimulus was paired with the CS+, the CS- and hand posture stimuli that ranged in functional similarity between the CS+ and CS- (generalisation stimuli). The primary outcome was participants' pain intensity ratings. The results showed that there was no difference in the pain ratings for the CS+ compared to those of the CS-. That is, there was no evidence of conditioning-induced hyperalgesia. Moreover, conditioned hyperalgesia did not decrease as the generalisation stimuli's similarity to the CS+ decreased. This study does not demonstrate that hyperalgesia can be a conditioned response. This study highlights that the choice of CS, US and rating scale, and the robustness of each, are critical determinants of a conditioning effect.

Introduction

Pain is a complex perceptual experience (Ballantyne & Sullivan, 2015; Tabor et al., 2015; Tabor, Thacker, Moseley, & Körding, 2017). We still do not fully understand why acute episodes of pain sometimes progress to chronic pain disorders. Factors such as injury type, psychological traits and occupation are perhaps intuitively sensible risk factors but have, at best, weak associations with the development of chronic pain (Chou & Shekelle, 2010; Griffioen et al., 2017).

Interest in the potential role of associative learning in chronic pain has recently been reignited (Moseley & Vlaeyen, 2015). Associative learning studies pair a neutral stimulus (conditioned stimulus; CS) with an aversive or a rewarding stimulus (unconditioned stimulus; US) which can result in the CS evoking similar responses (conditioned response; CR) to those evoked by the US (unconditioned response; UR; Gormezano, Kehoe, & Marshall, 1983). In the field of pain research, associative learning is recognised as the key process within the fear-avoidance model (Lethem, Slade, Troup, & Bentley, 1983), where pain is conceptualised as the US, movement as the CS, and avoidance as the response (Vlaeyen & Linton, 2000).

An important feature of associative learning, or 'classical conditioning', is stimulus generalisation, in which stimuli that share characteristics with the CS+ evoke similar CRs (Volders, Boddez, De Peuter, Meulders, & Vlaeyen, 2015). That is, the range of stimuli that can evoke the response becomes more general. In stimulus generalisation, the individual extrapolates knowledge about one event (e.g. 'when I see a big circle I receive a painful stimulus') to other similar events ('when I see a medium-sized circle, I may receive a painful stimulus'; Vlaeyen, 2015). Generalisation enables an individual to predict events with adverse potential, and therefore avoid them, and serves a protective function. Several studies (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015; Meulders, Harvie, et al., 2014; Meulders, Harvie, Moseley, & Vlaeyen, 2015; Meulders & Vlaeyen, 2012) have demonstrated that presenting participants with stimuli that are either functionally or perceptually similar to the CS+ evokes a response similar to that previously evoked by the CS+. The same principle applies to stimuli that are similar to the CS-. That is, the range of stimuli that cue for 'safety' becomes 'more general' (Vlaeyen, 2015). These similar stimuli are often referred to as generalisation stimuli (GS).

Recently, our group examined conditioning-induced hyperalgesia (Harvie, et al., 2016) and generalisation of pain expectancies in humans (Meulders, Harvie, et al., 2014). We found that a noxious US evoked more intense pain when it was paired with the CS+ than when it was paired with the CS- (i.e. conditioning-induced hyperalgesia; Harvie, et al., 2016). We also found that, in people with chronic unilateral hand pain, participants who learned to expect pain in association with a particular hand posture—the CS+— "generalised" by expecting pain with other, similar hand postures (Meulders, Harvie, et al., 2014).

Here, we aimed to determine whether a hand posture conditioning paradigm can also induce hyperalgesia, and whether that hyperalgesia generalises to other similar hand postures, as has been observed for pain expectancies (Meulders et al., 2015). We hypothesised that participants would rate a moderately painful laser stimulus more painful when the laser stimulus was preceded by the CS+ than when it is preceded by the CS- (conditioning-induced hyperalgesia). We also hypothesised that participants would show generalisation of hyperalgesia to similar hand postures, such that pain ratings of the moderately painful laser stimulus would decrease as the similarity between the generalisation stimuli and the CS+ decreased. Finally, several studies have highlighted the influence of affect on pain (Price, 2000; Wiech & Tracey, 2009) and generalisation (Meulders, Meulders, & Vlaeyen, 2014; Schechtman, Laufer, & Paz, 2010). As such, we explored the potential relationship between the extent of generalisation of conditioning-induced hyperalgesia, and affect and contingency awareness.

Methods

A priori protocol: The experimental protocol and analysis plan was locked and published online at the Open Science Framework (https://osf.io/3kvhx/). Any deviations from the protocol are noted as such.

Participants

Based on an *a priori* sample size calculation (effect size, f: 0.4; a power of 80% and $\alpha = 0.05$), 16 healthy participants were recruited using flyers, word of mouth and university

noticeboards. Participants were screened by telephone, and then in person prior to participation in the experiment. The following were exclusion criteria: presence of neurological diseases, vascular diseases, metabolic diseases, major or minor illnesses, acute or chronic pain, sensation problems, vascular problems (particularly Raynaud's phenomenon), psychiatric disorders or pregnancy. Additional exclusion criteria also assessed were: lack of proficiency in English, uncorrected problems with vision, left handedness, and participation in classical conditioning experiments within the last six months. Participants were screened for the following additional safety exclusion criteria: skin integrity of the right hand, unusual skin conditions (e.g. dermographism), and medication known to alter skin or temperature sensitivity. Finally, participants were monitored during the experiment via a video camera. Those who failed to look at the screen consistently or failed to provide 90% of the verbal ratings were excluded.

Ethics and consent

The experiment protocol was approved by the University of South Australia Human Research Ethics Committee (protocol number: 0000033772). Participants were informed that they would receive painful laser stimuli during the experiment and that they could withdraw from the experiment at any time without prejudice. All participants signed a consent form and were given an honorarium of AU$20 per hour.

Equipment

The experiment was programmed in Gentask (Compumedics Neuroscan, ElPaseo, TX) and run on a Windows XP (Windows XP Enterprise, 2009 Microsoft Corporation, Dell Inc., Australia) computer with 1.95 GB of RAM and an Intel Core 2 Duo I5-2400 CPU running at 3.10 GHz.

Stimuli (see Figure 1)

Conditioned, generalisation and distractor stimuli

Previous studies have shown that picture-specific motor imagery tasks can be used as conditioned stimuli (CS+ and CS-), generalisation stimuli (GS) and distractor stimuli (DS; Meulders et al., 2015). Given this precedent, we used images of distinct hand postures, ranging from fully flexed to fully extended, for the conditioned stimuli and generalisation

stimuli. The images allocated as CS+ and CS- were hand postures at extremes of flexion and extension, counterbalanced across participants. The GSs were images depicting novel hand postures that varied in perceptual similarity between the CS+ and CS-. Additional images of unrelated hand postures were used as distractor stimuli, in order to increase the difficulty of the task (see Figure 1 for images). Each CS hand posture image was presented in different orientations (two viewed from the medial aspect and two from the lateral), and was presented once during each experimental block in the Acquisition phase. Different orientations of the CSs were used to prevent the participants from becoming conditioned to the geometric features of the image, but rather to the hand posture itself (Meulders et al., 2015). Only one orientation of each GS and DS was displayed.

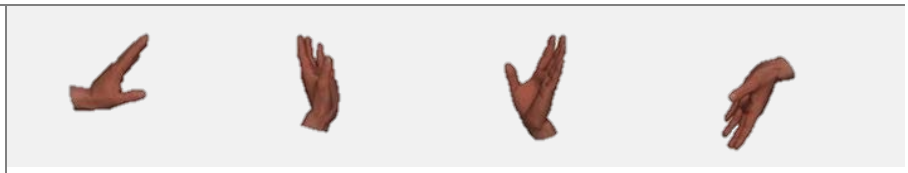
Stimulus type	Hand posture image
Conditioned stimuli – fully extended	
Conditioned stimuli- fully flexed	
Generalisation stimuli – range in similarity between CSs	
Distractor stimuli	

Figure 1. Hand posture images used for CSs, GSs and DSs. Each orientation is shown for the conditioned stimuli.

Unconditioned stimulus

The unconditioned stimulus (US) was a painful Nd:YAP laser stimulation (5 ms pulse duration, 4 mm spot diameter) delivered by a Stimul 1340 machine (Deka, Italy) to the dorsum of the right hand.

Sequencing of the conditioned and generalisation stimuli

Previous research (Struyf, Zaman, Vervliet, & Van Diest, 2015; Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015) has highlighted the role that perceptual discrimination can have in stimulus generalisation. Therefore, an e-survey was conducted prior to this experiment to verify that respondents used the same strategy we used to sequence the hand images—a gradual progression of the hand postures from fully flexed to fully extended. The survey findings showed that 80% of all respondents (120 of 150) sequenced the hand images the same way we did, and reported the same strategy as we used.

Group allocation and hand posture image randomisation

The order of the hand posture images in each block of trials was randomised, with a restriction that no two consecutive trials included the same CS (e.g. a fully flexed hand). To minimise the potential that the results were due to an ordered effect of the images in each block, participants were randomised *a priori* to receive: three of nine semi-randomised blocks for acquisition; one of two semi-randomised blocks for Generalisation US expectancy; one of two semi-randomised blocks for Generalisation pain intensity; and two of four semi-randomised blocks for extinction.

Outcomes and measures

Rating scales

Numerical rating scales (NRS) were used during the experiment and calibration. Participants used an 11-point NRS to rate pain intensity in response to the question, "How painful was the stimulus?" The anchors were 0 - "no pain", and 10 - "the worst pain tolerable".

This study considered contingency awareness and expectancy awareness as two distinct constructs (see Lovibond & Shanks, 2002 for a full review). Expectancy awareness was analysed and used as a manipulation check, while contingency awareness was explored as a variable in the generalisation of conditioning-induced hyperalgesia.

Manipulation checks

Participants used an 11-point NRS to rate US expectancy (the likelihood of pain) in response to the question, "How much do you expect the painful stimulus?" The anchors were 0 - "not at all" and 10 – "very much". At the end of each experimental block, participants used the 11-point NRS to rate contextual fear in response to the question, "How fearful are you?" The anchors were 0 - "not at all" and 10 - "very much".

Post-experiment tests and ratings

Contingency awareness: Following the Extinction phase, participants were presented with an image of each hand posture (CS+, CS- and GS 1-6) and asked to identify the images that were most and least likely to be associated with the painful US. Participants who identified the

CS+ or GS6 as being *most* likely to be associated with the painful US, *and* rated the CS- or GS1 as *least* likely to be associated with the painful US, were considered to be 'contingency aware'. Participants who were unable to identify the hand posture images most and least likely to be associated with the painful US were considered to be 'contingency unaware'.

Blinding

Participant naivety was assessed by asking participants the question, 'What do you think this study was testing?'

Affective differences

Participants completed the Positive and Negative Affect Scale (PANAS; Crawford & Henry, 2004), Catastrophising Assessment Tool (CAT; Lefebvre, Keefe, Caldwell, Waters, & Ulrich, 2002) and State-Trait Anxiety Inventory (STAI; Spielberger, 1989) questionnaires. These questionnaires allowed exploration of the influence of high (versus low) positive affect and high (versus low) negative affect, high (versus low) catastrophising, and high (versus low) anxiety on our outcomes, but these exploratory analyses serve to generate hypotheses rather than test them. After each questionnaire, the participants with the five highest scores in each trait were allocated to either the high positive affect (PA) group, high negative affect (NA) group, or high catastrophising (CAT) group. Conversely, the participants with the five lowest scores were allocated to the low PA group, low NA group, or low CAT group.

Experimental Procedure

Preparation

Participants

Participants were seated in a comfortable armchair, 600 mm from a screen in a dimly-lit room. Each participant's right hand was shielded from view and placed on a foam cushion. A 70 mm square was marked on the dorsum of the right hand. The laser beam's diameter and focus were calibrated before each participant. The laser hand piece was 150—200 mm from the dorsum of the hand, depending on hand thickness (this was factored into the calibration procedure).

Two researchers conducted the experiment from a room adjacent to the participants' room. The first researcher operated the Nd:YAP laser and Gentask software. Each experimental block was pre-programmed using Gentask software (Compumedics Neuroscan, El Paseo, TX). The Gentask software controlled the delivery of the images (displayed on the participant's screen) and the laser stimulation (via transistor-to-transistor logic). The second researcher (who was blinded to the experimental hypotheses) recorded the participant's verbal ratings and operated the CNC Mac 3 software (Solid Edge ST 8, CNC Mac 3 controller program, Australia). This software controlled the robotic arm to which the laser hand piece was attached. The software had pre-programmed coordinates with an 8 mm spacing, to which the robotic arm would move the laser hand piece after each stimulus. The stimulation paradigm was adapted from existing recommendations for the use of experimental laser stimuli, with particular emphasis on moving the laser after each stimulus delivery, and allowing at least 30 s between stimuli and 3—8 minutes between blocks (Madden et al., 2016).

Each participant's hand was visually monitored via video camera and the skin temperature was checked with a non-contact infrared thermometer before and after experimental blocks. Detection of a temperature difference greater than 1°C between the non-stimulated and stimulated hand was used as a prompt to extend the inter-block interval (Mancini et al., 2013).

Calibration phase

A single series of laser stimuli with ascending energy outputs was used to determine the intensities needed to elicit pain ratings of 3 out of 10 and 5-6 out of 10. To ensure consistency in these ratings, the same energy stimulus was administered to three different locations. The intensity was considered acceptable if it elicited the target rating at at least two of the three locations. If the ratings were not accepted, then the stimulus intensity was adjusted accordingly, and the process repeated until consistent pain ratings were achieved at both intensity levels. The 5-6/10 rating was assigned as the highly painful US, and was paired with the CS+ during the Acquisition phase and Generalisation phase - *US expectancy*. The 3/10 rating was assigned as the moderately painful US, and was paired with the CS+, CS- and GS during Generalisation phase - *pain intensity*. Five participants were excluded and subsequently replaced because they failed to report the maximum safe laser stimulus (3.25 J) as painful.

Main procedure

A within-subjects experimental design was combined with a classical (Pavlovian) trace conditioning paradigm. The study comprised a single 60-minute session with each participant. In order to undertake the manipulation check (and replicate the effect shown in a previous study in people with hand pain but without noxious stimuli; Meulders et al., 2014), we assessed both the *expectancy* of pain and the *intensity* of pain. Table 1 shows the different blocks of the experiment, the trial types included in each block, the number of trial types presented in each block, the type of rating that was required in each block and the number of times each rating was required in each block. Fear ratings were only measured at the end of each experimental block.

Table 1: Outline of the trials and ratings required for each experimental block.

Block	Trials included									Ratings required		
	CS+ US HIGH	CS+ US MOD	CS+ALONE	CS- US MOD	CS- ALONE	GS 1-6 US MOD	GS 1-6 ALONE	DS US MOD	DS ALONE	PAIN INT.	USEXP	FEAR
	Number of trials									Ratings		
Acquisition 1	4				4				8		8	1
Acquisition 2	4				4				8		8	1
Acquisition 3	4				4				8		16	1
Generalisation US expectancy	2				2		1x6 GSs		2		12	1
Generalisation Pain intensity		2		2		1x 6 GS		2		12		1
Extinction 1			4		4				8		16	1
Extinction 2			4		4				8		16	1

Note: Table segments are shaded if, during a block, a particular CS or DS or GS occurred and a particular conditioned response was recorded.

CODE	EXPLANATION
US	unconditioned stimulus
CS+ US HIGH	CS+ hand posture image paired with the highly painful US
CS+ US MOD	CS+ hand posture image paired with the moderately painful US
CS+ALONE	CS+ hand posture image without the US
CS- US MOD	CS- hand posture paired with the moderately painful US
CS- ALONE	CS- hand posture image without the US
GS 1-6 US MOD	The six-different generalisation hand posture images paired with the moderately painful US
GS1-6 ALONE	The six-different generalisation hand posture images without the US
DS US MOD	The distractor hand posture stimuli paired with the moderately painful US
DS ALONE	The distractor hand posture stimuli without the US
PAIN INT	Pain intensity NRS rating
USEXP	US expectancy NRS rating

Acquisition phase

The Acquisition phase consisted of three blocks of 16 trials (Table 1). US expectancy ratings followed 50% of trials in Acquisition blocks one and two, and 100% of trials in Acquisition block three. This was done so as to prepare the participants for the Generalisation phase and to prevent an obvious transition from the acquisition to the Generalisation phase. All

CS+ trials were reinforced with the highly painful US (100% reinforcement rate). The trial flow for Blocks 1, 2 and 3 is shown in Figure 2.

Generalisation phase – US expectancy

This Generalisation phase consisted of one block of 12 trials, each followed by a US expectancy rating. The trial flow was the same as the final block in the Acquisition phase, except for trial types (Table 1). Once again, only the CS+ was paired with the highly painful US. The trial flow for this block is shown in Figure 2.

Generalisation phase - pain intensity

This Generalisation phase consisted of one block of 12 trials, each followed by a US expectancy rating. All visual stimuli (CS+, CS- and GS) were followed by the moderately painful US that had been previously rated at 3/10 during calibration (Table 1). The participants rated pain intensity using the 11-point NRS. The trial flow differed from the Acquisition phase and Generalisation phase *US expectancy* (Figure 2).

Extinction phase

The Extinction phase consisted of two blocks of 16 trials, each followed by a US expectancy rating (Table 1). The Extinction phase was similar to the third Acquisition block except that the CS+ was no longer followed by the highly painful US – it was presented unpaired (Figure 2).

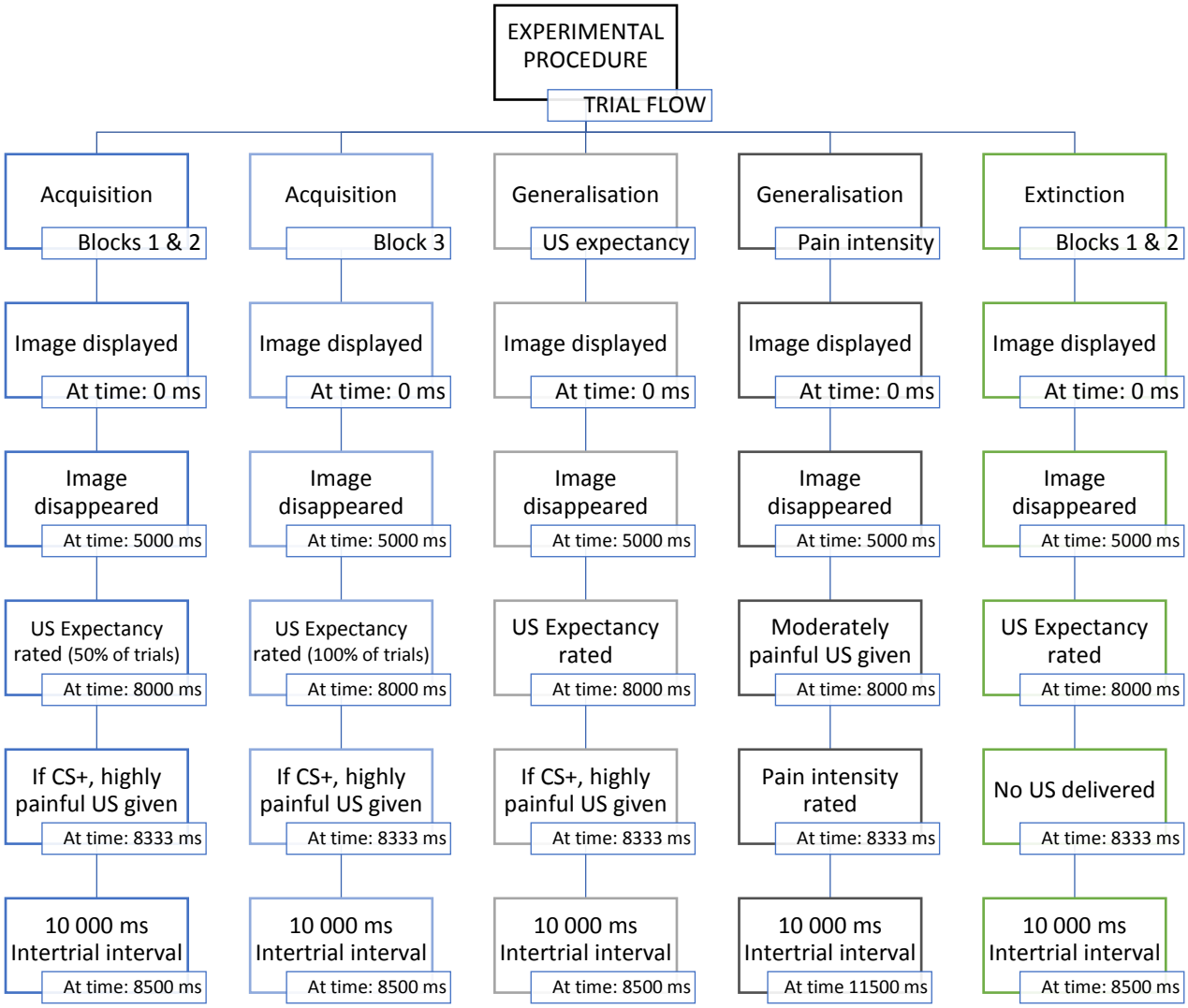


Figure 2. A flow chart of the trial procedure for each experimental block.

Statistical Analyses

General approach

Data analyses was planned *a priori* and locked in the Open Science Framework in November 2016. All data were entered into Microsoft Excel and then analysed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). The data distributions were checked via visual examination of Q-Q plots and use of the Shapiro-Wilk statistic. Skewness was assessed using the distribution skewness statistic.

Parameters

After consultation with a statistician, the following parameters were set:

1) if the data were not normally distributed, and the skewness statistic was not between -1 and 1, then non-parametric tests would be used; 2) regarding repeated measures ANOVA: if

Mauchly's test indicated that the assumption of sphericity had been violated, then Greenhouse-Geisser estimate adjusted values would be reported; 3) regarding generalisation of conditioning induced hyperalgesia: a CS+ vs. CS- difference was a prerequisite for assessing for generalisation. That is, the response to the CS+ must be greater than that to the CS-, to proceed with testing for generalisation; 4) regarding independent *t*-tests: if Levene's test statistic was significant, then the null hypothesis—that the variance between the groups was equal—would be rejected (Field, 2009).

Manipulation check US expectancy

To determine the influence of hand postures (generalisation stimuli, GSs) on US expectancy ratings, we conducted a one-way repeated-measures ANOVA with eight levels (hand postures; CS+, GS1-GS6 & CS-). Simple contrast analyses were used to compare US expectancy for each of the seven hand posture images (CS+, GS1-GS6) to US expectancy for the CS- hand posture image.

Primary Analysis

Our primary aim was to determine whether conditioning-induced hyperalgesia was elicited, and whether it showed generalisation. A prerequisite was a demonstration of differentially-conditioning induced hyperalgesia to CS+ and CS-, which we tested for using a paired *t*-test. If the *t*-test was significant ($p < 0.05$), a one-way repeated measures ANOVA with six levels (hand posture images GS1- GS6) was conducted to test for generalised hyperalgesia.

Secondary Analysis

Contingency awareness and generalisation of conditioned hyperalgesia: Our secondary aim was to determine the extent to which contingency awareness modulated conditioning-induced hyperalgesia to similar hand postures (GSs). A two-way repeated measures ANOVA of pain intensity ratings with a between-group factor (aware and unaware) and a within-subject factor of hand posture (GS1-GS6) was conducted.

Exploratory Analyses

Affective Differences: We wanted to explore the relationship between state affect and conditioned and generalised hyperalgesia. Because these were exploratory analyses, we did not correct for multiple measures. No adjustments of participants' raw scores for each questionnaire (PANAS, STAI and CAT) were made prior to analysis. A two-way repeated measures ANOVA of pain intensity ratings was conducted with a between-group factor (2: high group, low group) and a within-subjects factor of hand posture (8: CS+, CS- and GS1-GS6).

Contingency awareness on generalisation of US expectancy: An exploratory analysis investigated whether a difference in contingency awareness (aware versus unaware) modulated US expectancy ratings. A two-way mixed-method ANOVA was conducted with a between-group factor (aware and unaware) and a within-subjects factor of hand posture (CS+, GS1-GS6 and CS-).

Deviation from our a priori protocol

In line with best practice recommendations for transparency and replicability in science (Nosek et al., 2015), we posted our full *a priori* protocol online (https://osf.io/3kvhx/), where it was 'locked'. The following changes were made to the analytical approach after the protocol had been locked: (1) we had taken an *a priori* decision that a CS+ vs. CS- difference must be demonstrated as a prerequisite for generalisation assessment. However, based on the orienting response literature, we decided to analyse whether conditioning-induced hyperalgesia was elicited by similar but novel hand postures (GSs) even though we had not found a difference between CS+ vs. CS- pain intensity ratings (Esber & Haselgrove, 2011). The orienting response (when an organism attends/orients to the stimulus; Kaye & Pearce, 1984; Swan & Pearce, 1988), coupled with theories on stimulus predictability and uncertainty (Esber & Haselgrove, 2011), suggests that the salience of a cue that predicts a reinforcer with a measure of uncertainty will be greater than the salience of a cue that predicts a reinforcer with certainty. The current study used continuous reinforcement, so the salience of (and therefore the response to) the CSs may have been reduced after repeated exposure. However, according to this framework, the salience of, and responses to, the GSs would not be attenuated because the GSs were novel.

Results

Descriptive data for participants

Participants excluded and replaced: A total of 22 healthy participants were recruited for this study. Five participants were excluded because they failed to report the maximum safe laser stimulus (3.25 J) as painful. One participant was excluded following a manipulation check for failure to provide 90% of the verbal ratings. The remaining 16 healthy participants (6 females and 10 males), aged between 20—58 years old (mean ± SD: 27 ± 9.3) participated in and completed this experiment. Hand temperature*:* The mean recorded temperature for all 16 participants' right hand was 30.45°C (SD: 1.58°C, range: 27—32°C).

Rating of unconditioned stimulus

The mean laser stimulus intensity that elicited a 5-6/10 pain intensity rating was 2.26 Joules (SD: 0.44, range: 1.5—3.0 J). For a pain intensity rating of 3/10, the mean laser intensity was 1.84 J (SD: 0.42 J, range: 1.25—2.50 J).

Blinding: Analysis of participants' responses to the blinding question showed that all participants were blind to the main aim of this study. That is, no participant identified the study's hypothesis.

Testing of assumptions

Manipulation check: A Shapiro-Wilk test statistic of 0.308 ($p < 0.001$) indicated that the US expectancy data were not normally distributed. However, the distribution skewness test statistic of 0.554 was within the acceptable range, as defined *a priori*. Therefore, parametric tests were used. Primary and secondary outcome: A Shapiro-Wilk test statistic of 0.927 ($p = 0.00$) indicated that the pain intensity ratings data were not normally distributed. However, the distribution skewness test statistic of 0.765 was within the acceptable range, as defined *a priori*. Therefore, parametric tests were used.

Exploratory outcomes

Affective differences: Shapiro-Wilk statistics of 0.884 ($p = 0.044$) and 0.861 ($p = 0.020$) for the PANAS and STAI questionnaires, respectively, indicated that the negative affect scores were not normally distributed. However, the distribution skewness test statistics of 0.451 and 0.564, respectively, were within the acceptable range, as defined *a priori*. Therefore,

parametric tests were used. Shapiro-Wilk statistics of 0.896 (p = 0.070) and 0.891 (p = 0.058) for the PANAS and CAT questionnaires, respectively, indicated that the positive affect scores were normally distributed.

Contingency awareness and generalisation of US expectancy: A Shapiro-Wilk statistic of 0.308 (p < 0.001) indicated that the US expectancy data were not normally distributed. However, the distributed skewness test statistic of 0.554 was within the acceptable range defined *a priori*. Therefore, parametric tests were used.

Manipulation check (US expectancy awareness)

Mauchly's statistic ($X^2_{(27)}$ = 43.06, p = 0.05) indicated that sphericity had been violated; therefore Greenhouse-Geisser estimates are reported. US expectancy ratings differed by hand posture image (main effect of image, $F_{(3.4, 52.3)}$ = 26.2, p = 0.01). In particular, US expectancy was rated higher for the CS+ than for the CS- ($F_{(1, 15)}$ = 58.1, p = 0.00), indicating that participants had learned that the CS+ was associated with the highly painful US and the CS- was not (see Figure 3). US expectancy was also rated higher for the GS6 ($F_{(1, 15)}$ = 12.02, p = 0.003) and GS5 ($F_{(1, 15)}$ = 118.6, p = 0.00) than for the CS-, which is evidence for generalisation of expectancy.

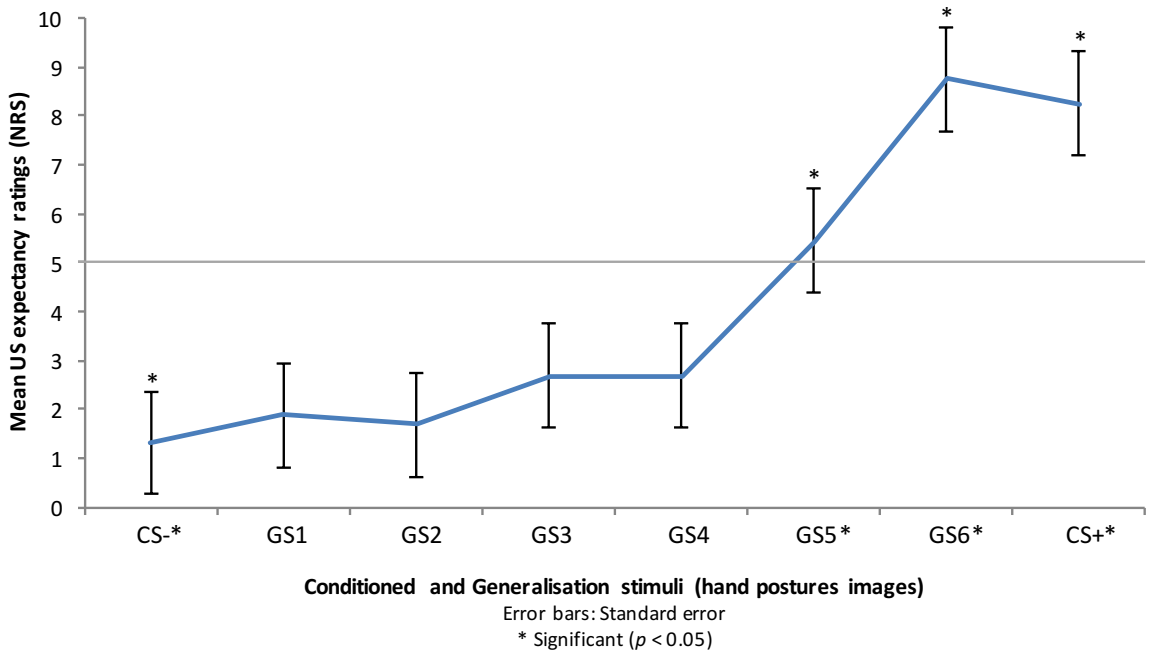


Figure 3. Generalisation of mean US expectancy ratings for the different hand posture images.

Primary Analysis

Mauchly's test statistic (W = 0.007, p < 0.001) indicated that sphericity was violated, therefore Greenhouse-Geisser-adjusted statistics are reported. There was no difference in pain intensity ratings between the CS+ and the CS- trials, during the test phase ($t_{(15)}$ = 0.000, p = 0.5). Hence, there was evidence that there was no classical conditioning effect on pain ratings at the group level. *Post hoc* analysis of conditioning-induced hyperalgesia to the GSs used a repeated measures ANOVA with eight levels (CS+, CS- and GS1 - 6). The pain intensity ratings did not differ for the different novel hand postures ($F_{(4, 58)}$ = 0.492, p = 0.735; Figure 4). Simple contrasts were used to assess for a difference between pain intensity ratings for each GS compared to pain intensity ratings for the CS- (used as baseline). There was no difference in pain intensity ratings for each GS trial compared to the CS- trial (GS1: $F_{(1,15)}$ = 0.35, p = 0.57; GS2: $F_{(1,15)}$ = 0.52, p = 0.48; GS3: $F_{(1,15)}$ = 0.46, p = 0.51; GS4: $F_{(1,15)}$ = 0.83, p = 0.38; GS5: $F_{(1,15)}$ = 0.05, p = 0.82; GS6: $F_{(1,15)}$ = 0.80,p = 0.78).

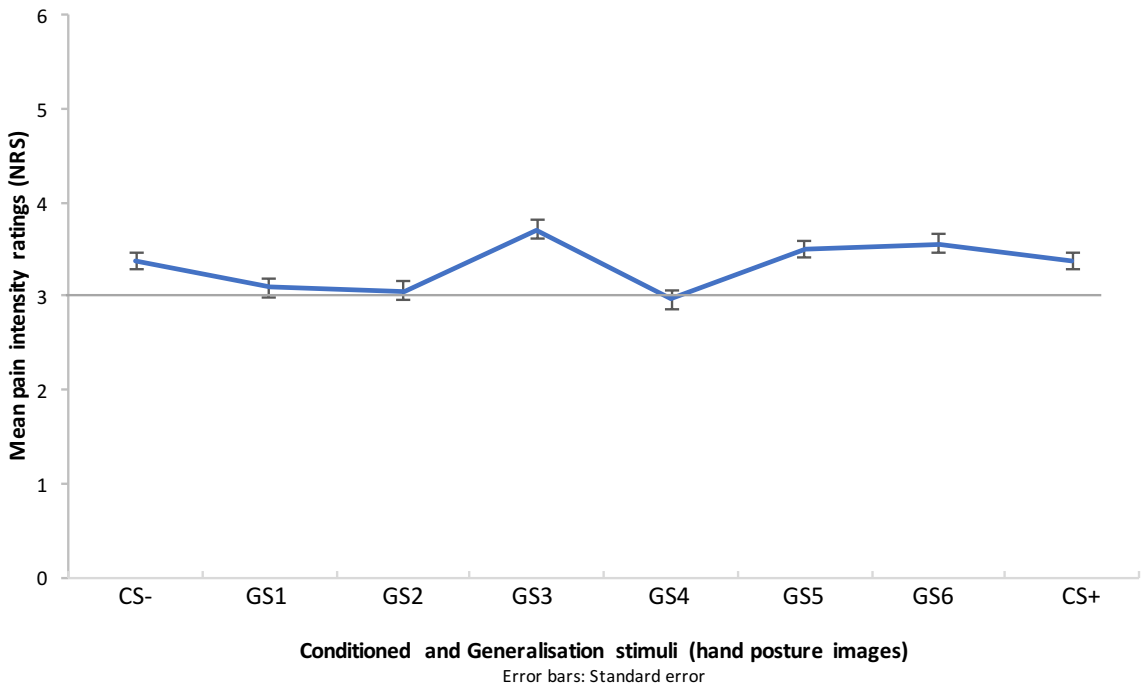


Figure 4. Generalisation of mean pain intensity ratings to the different hand posture images.

Secondary Analysis

Contingency awareness and generalisation: There was no effect evident from the primary analysis. Therefore, an exploratory variable was added to determine whether an effect

emerged when we accounted for contingency awareness. Levene's test statistic was non-significant (F = 0.107, p = 0.748); therefore, equal variance between the groups was assumed. There was no difference between the two contingency groups (aware and unaware) in pain intensity ratings between the CS+ and CS- trials ($F_{(1, 14)}$ = 0.674, p = 0.426; Figure 5).

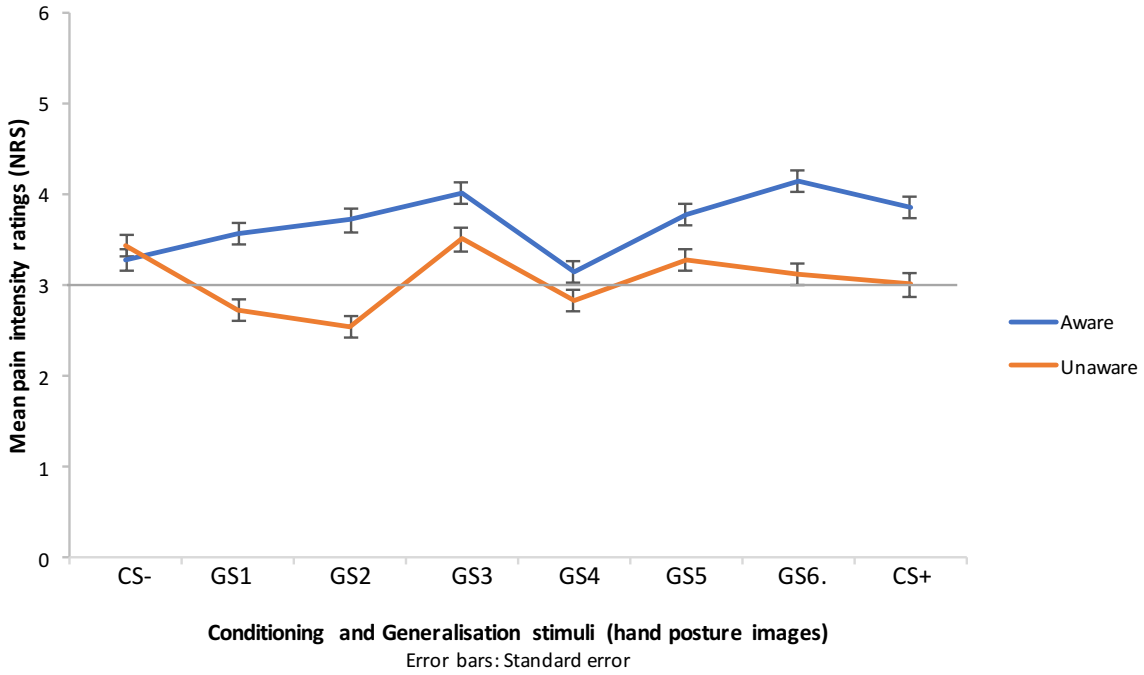


Figure 5. Contingency effects on the extent of generalisation of mean pain intensity ratings.

Exploratory Analysis

Analysis of affective differences: Mauchly's test statistics for the NA and STAI groups were W = 0.001, p = 0.109 and W = 0.001, p = 0.083, respectively, indicating that sphericity was not violated. Mauchly's test statistics for the PA and catastrophising groups were W = 0.000, p < 0.001 and W = 0.000, p = 0.009, respectively, indicating that sphericity was violated, so Greenhouse-Geisser-adjusted statistics are reported. For all three questionnaires, the difference in pain intensity ratings between CS+ trials and the CS- trials was not affected by grouping (High vs. Low NA group: $F_{(1,8)}$ = 4.227, p = 0.074; Figure 6a; High vs. Low PA group: $F_{(1,8)}$ = 0.032, p = 0.862; Figure 6b; High vs. Low catastrophising group: $F_{(1,8)}$ = 0.004, p = 0.953; Figure 6c; High vs. Low Negative STAI group: $F_{(1,8)}$ = 1.766, p = 0.220; Figure 6d).

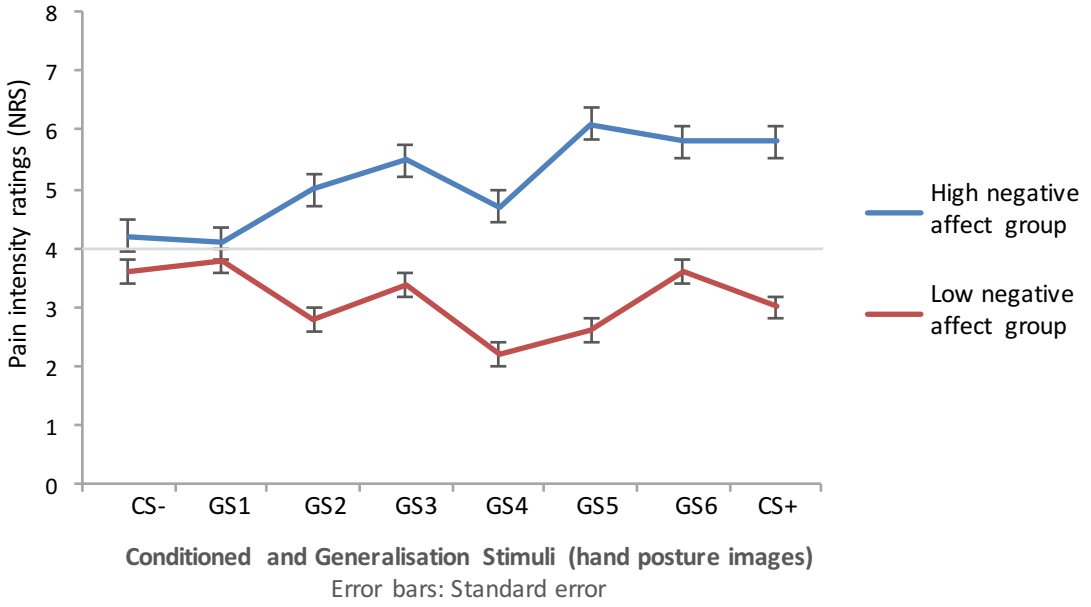


Figure 6a. Comparison of High and Low negative affect groups' generalisation of mean pain intensity ratings for the CS and GS.

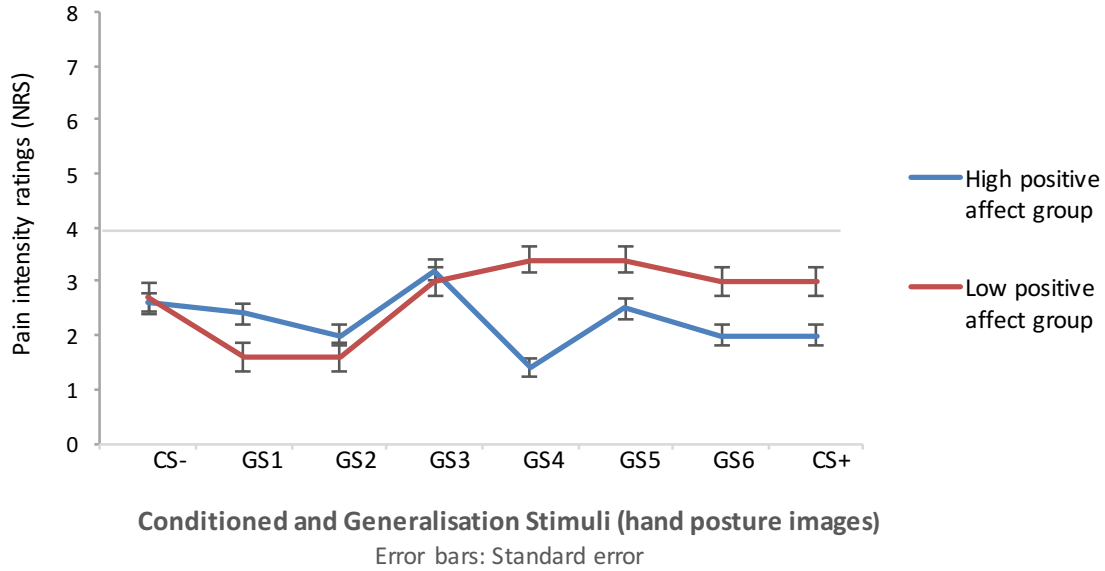


Figure 6b. Comparison of High and Low positive affect groups' generalisation of mean pain intensity ratings for the CS and GS.

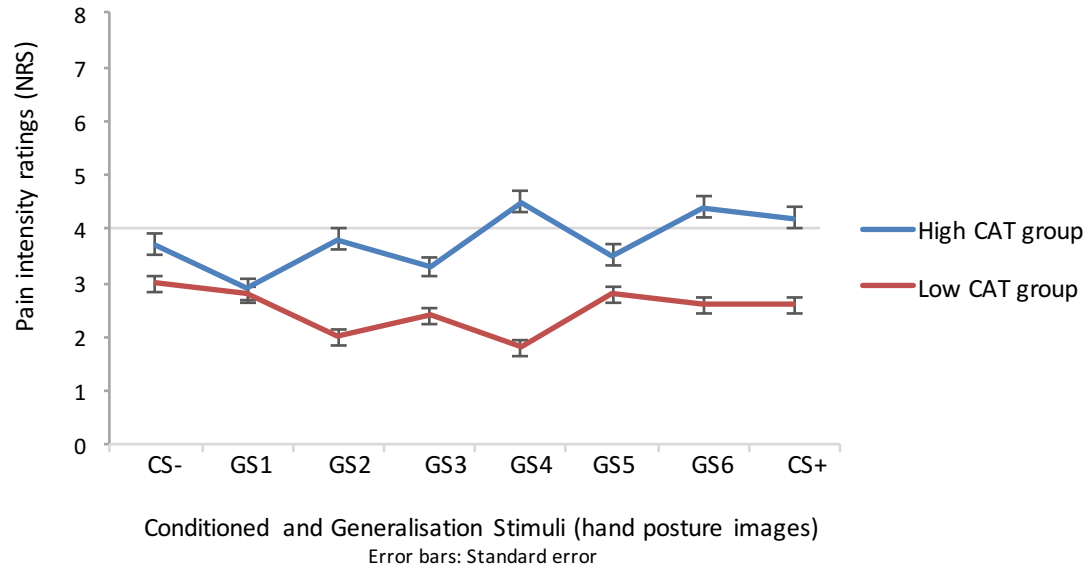


Figure 6c. Comparison of High and Low castastrophising groups' generalisation of mean pain intensity ratings for the CS and GS.

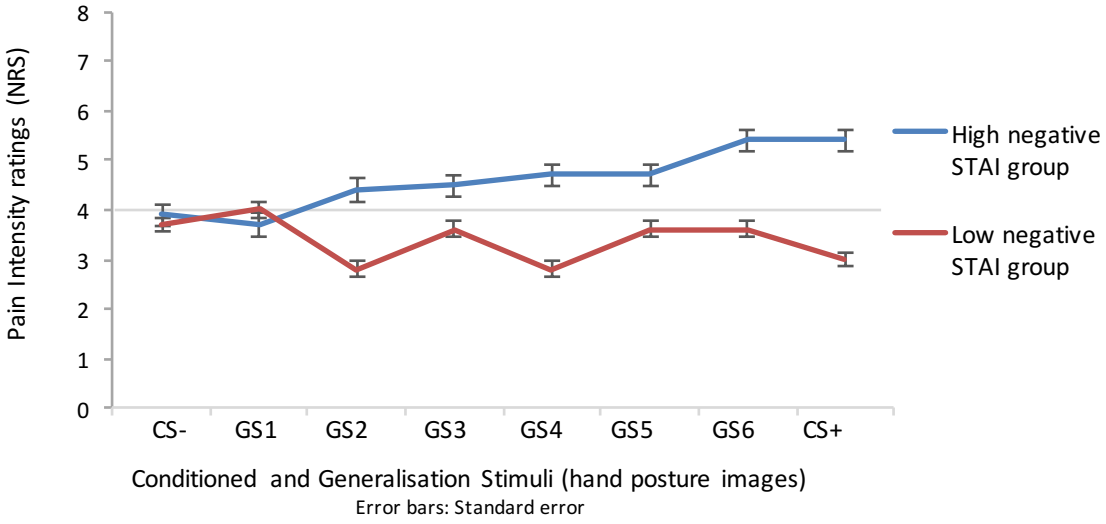


Figure 6d. Comparison of High and Low negative STAI groups' generalisation of mean pain intensity ratings for the CS and GS.

Influence of contingency awareness on US expectancy ratings for the GSs: Mauchly's statistic ($\chi^2_{(27)}$ = 35.5, p = 0.15) indicated that the assumption of sphericity had been met. Contingency-aware participants rated the US expectancy for CS+ higher than the contingency-unaware participants (main effect: $F_{(7, 98)}$ = 3.816, p < 0.001; for CS+: $F_{(1, 14)}$ = 19.873, p < 0.001), and the CS- lower than the contingency-unaware participants ($F_{(1, 14)}$ =10.04, p < 0.007; Figure 7). Contingency-aware participants also rated US expectancy lower than contingency-unaware participants for the following hand postures: GS1 ($F_{(1, 14)}$ = 10.228, p = 0.006), and GS2 ($F_{(1, 14)}$ = 14.412, p = 0.002). Individual participant results for the effect of the strength of contingency awareness on US expectancy ratings are shown in the Supplementary File.

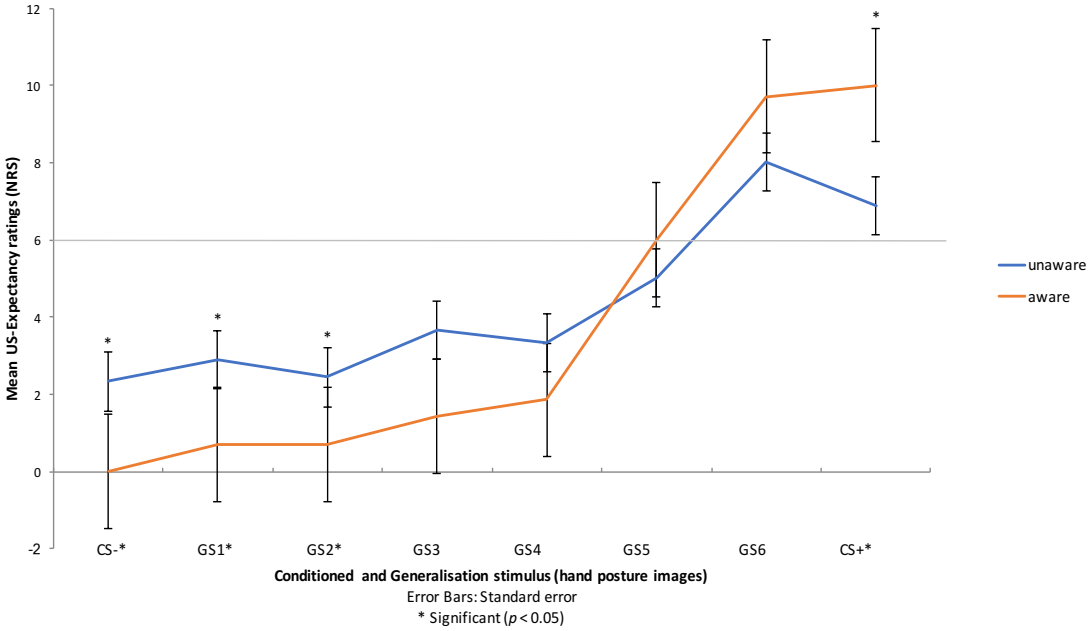


Figure 7. The effects of contingency awareness on the extent of generalisation of US expectancy.

Sequencing of stimuli: All participants provided a sequence that matched our experimental sequence of hand posture images, i.e., a gradual progression from fully extended to fully flexed, consistent with our pilot tests and survey.

Transparency and openness: All data are available from the authors on request.

Discussion

We hypothesised that participants would rate a moderately painful laser stimulus as more painful when the laser stimulus was preceded by the CS+ than when it was preceded by the CS- (conditioning-induced hyperalgesia). Also, that participants would show generalisation of hyperalgesia to similar hand postures, such that their pain ratings of the moderately painful laser stimulus would decrease as the similarity between the generalisation stimuli and the CS+ decreased. Our results did not support our hypotheses: there was no difference in pain ratings of the moderate laser stimulus, regardless of whether it was preceded by the CS+ or the CS-. That is, our classical conditioning paradigm did not induce hyperalgesia, which also meant that our hypothesis regarding the generalisation of conditioning-induced hyperalgesia was not supported.

These findings were unexpected, and add to a complex and contrasting literature on classical conditioning and pain. Our conditioning paradigm clearly led to predicted changes in US expectancy, which also generalised to similar but novel hand posture images. This corroborates previous work (Meulders & Vlaeyen, 2012, 2013) and extends it by showing that we can replicate the responses shown by people with hand pain (Meulders et al., 2015) when we induce hand pain experimentally in healthy volunteers. Critically, however, this difference in US expectancy did not translate into conditioning-induced hyperalgesia.

Our failure to induce hyperalgesia contrasts with previous studies that used similar protocols but different stimuli (Jenewein et al., 2013; Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015; Jensen et al., 2012, 2014; Ploghaus et al., 2001). This raises the possibility that the CS types used in human conditioning experiments should be ranked hierarchically, according to their effects on participants' motivational states (Itti, 2005). That is, studies that have used faces have demonstrated larger effect sizes for conditioning-induced hyperalgesia (Jensen et al., 2012, 2014) than studies using an arbitrary CS (Domjan, 2005), such as hands (current study) or shapes (Ploghaus et al., 2001). Perhaps this is because faces carry more inherent meaning (Reicherts, Gerdes, Pauli, & Wieser, 2013) or social relevance (Karos, Meulders, & Vlaeyen, 2015a; Karos, Meulders, & Vlaeyen, 2015b). Another possible explanation for our null effect may relate to a difference between the hand images that we used and the participants' own hands. Stronger effects tend to be observed in conditioning studies when

the stimulus features are more salient or 'recognisable'. By using generic hand models, we may have missed an effect that would have been present if images of the participants' own hands were used (Hommel, 1997; Navon, 1977; Sripati & Olson, 2009).

The difference in the temporal relationship between CS and US (Domjan, 2000; Lavond & Steinmetz, 2003) may also have limited a conditioning effect. The 3-second trace interval used in the current study contrasts from the 0-second interval used by studies that successfully demonstrated conditioning-induced hyperalgesia (Jenewein et al., 2013; Jensen et al., 2012, 2014, 2015; Ploghaus et al., 2001). Perhaps the longer trace interval used in this study hampered the formation of associations and precluded a conditioning effect (Domjan, 2000, 2014). We suspect this is unlikely, however, because we did find evidence of conditioned generalisation of US expectancy, replicating the results of a study that used 0-second trace intervals (Meulders et al., 2015).

The use of continuous reinforcement may have reduced the salience of the CS, resulting in the association between the CS and US becoming extinguished before it could be detected in the testing phase. The idea that predictability and uncertainty play key roles in determining stimulus salience is largely accepted in the psychology literature (Esber & Haselgrove, 2011; Hogarth, Dickinson, Austin, Brown, & Duka, 2008; Wills, Lavric, Croft, & Hodgson, 2007). There is evidence that the conditioned response is better maintained when the CS is an inconsistent predictor of the US (partial reinforcement), than if it is a consistent predictor of the US (continuous reinforcement; Swan & Pearce, 1988).

We may have reduced the sensitivity of our design to detect conditioning by our selection of an 11-point NRS instead of a 101-point NRS, as used in previous conditioning-induced hyperalgesia studies (Arntz, Spit, & Merckelbach, 1997; Jenewein et al., 2013; Jensen et al., 2012, 2015). A recent study by our group (Harvie et al., 2016) reported a consistent effect of CS+ that was, for some participants, as small as 5 mm on a 150 mm scale. We based our choice on the effect sizes reported in previous studies, but we cannot exclude the possibility that a more sensitive scale may have revealed different results. In any case, however, we would expect any effects to be very small, which casts doubt over their clinical relevance, should they exist.

Our exploratory analyses generated two interesting hypotheses. First, that affect might influence generalisation of conditioning-induced hyperalgesia. That is, participants in the high negative affect group had higher pain intensity ratings for all the hand posture stimuli than the low negative affect group. Second, that contingency awareness of the association between the CS+ and the highly painful US might influence generalisation of pain expectancies. The preliminary evidence was that unaware participants had an up to 30% greater chance of expecting that even the CS- would be painful, compared to their aware counterparts. These results are relevant to the findings of two studies (Meulders et al., 2014; Meulders, Meulders, Stouten, De Bie, & Vlaeyen, 2017). The latter study (Meulders et al., 2017) showed that in comparison to healthy controls, patients with fibromyalgia (FM) failed to identify safety cues (that the CS- was predictive of no US), which resulted in flatter generalisation gradients. The former study (Meulders et al., 2014) showed similar findings—of a deficit in safety learning in patients with chronic unilateral hand pain. The finding that a difference in awareness affects the generalisation of US expectancy is consistent with the imprecision hypothesis of chronic pain (Moseley & Vlaeyen, 2015). That is, perhaps, being unaware of the difference between the CS+ and CS- reflects imprecise encoding of the initial painful event (CS+ paired with the painful-US), thus resulting in greater generalisation.

Strengths and limitations

A clear strength of the current study is that we complied with published standards for transparency and openness (Nosek et al., 2015) by pre-registering the study protocol and the study analysis plan, fully disclosing unforeseen deviations from the protocol, calculating a sample size based on predicted and clinically-relevant effects *a priori*, blinding the researcher responsible for data entry to our hypothesis, and testing whether patients were blinded to the study hypotheses. Although these aspects are highly recommended, they are almost never implemented. We also attempted to clarify the issue of perceptual discrimination between the proposed CS and the GSs in two ways (Struyf et al., 2015; Vervliet & Van, 2015). First, the sequence of the hand posture images we used was found to conform to the intuitive sequencing approach of 80% of participants in a separate *a priori* 150-person e-survey (Talbot, Madden, Jones & Moseley, unpublished). Second, each participant was asked to place the hand posture images into a sequence at the completion of the experiment and this, again, matched the sequence on which we based generalisation stimuli. A limitation is that we did not assess participants' perceptual acuity throughout the

study, so cannot exclude the possibility that this changed (Vervliet & Van, 2015). Moreover, we did not assess whether participants could distinguish between the different hand postures. Even though we assessed the sequencing of the hand posture images, we cannot assume that participants did distinguish among the different hand posture images during the experiment. That we used different orientations for CS+ and CS- during the Acquisition phase may have reduced the number of paired CS trials. If the various orientations of the CS were perceived as different CSs, this study had only four CS+ and four CS-, rather than 12 CS+ and CS- trials, with which to learn the CS+/ US association. Finally, the method used to assess contingency awareness in this study may be susceptible to variation in reporting (Weidemann, Satkunarajah, & Lovibond, 2016) and the lack of continuous assessment of contingency awareness may have reduced the reliability of the findings, although both of these measures come with logistical challenges that we considered too large to overcome.

Implications

The current study raises several important implications for future research in this field. Our results strengthen previous assertions that pain expectancies are modulated by conditioning and generalisation (Harvie et al., 2016; Lissek et al., 2008; Madden et al., 2016; Meulders et al., 2015). This is relevant to clinical practice, particularly the fear-avoidance model (FAM), because what people expect changes their behaviour (Leeuw et al., 2007; Vlaeyen, Crombez, & Linton, 2016; Vlaeyen & Linton, 2012). This is perhaps also relevant to Bayesian modelling, which stipulates a role of expectation in perceptual processing (Tabor, Catley, Gandevia, Thacker, & Moseley, 2013). Perhaps the most obvious implication of the current work is that the role of associative learning in human pain conditions is not straightforward. Our own group have shown conditioning-induced hyperalgesia in some paradigms (Harvie et al., 2016; Madden, Harvie et al., 2016) and not in others (Harvie, Meulders et al., 2016). Other researchers have shown conditioning-induced hyperalgesia (Jensen et al., 2012, 2015; Miguez, Laborda, & Miller, 2014) and other researchers have argued against it (Arntz, 1991; Bräscher, 2014). This apparent confusion is reflected in clinical opinions - 98% of clinicians believed there is good evidence to show pain can be a conditioned response (Madden & Moseley, 2016), although some argue it is theoretically impossible (e.g. Quinter, 2016), and a systematic review (Madden, Harvie, et al., 2016) clearly showed there is only limited evidence to support either position. Clearly, more research is required to discover whether

associative learning contributes to persistent pain and, if so, how, and under what conditions.

Conclusion

We found no evidence of conditioning-induced hyperalgesia, but we found predictable patterns of conditioning-induced pain expectations. Our results suggest that the choice of CS and US, the rating scale used, and the robustness of the experimental design, might be critical determinants of whether pain or hyperalgesia can be observed to be 'conditioned'. This study highlights the need for rigorous assessment of perceptual discrimination and contingency awareness when investigating conditioning effects and generalisation.

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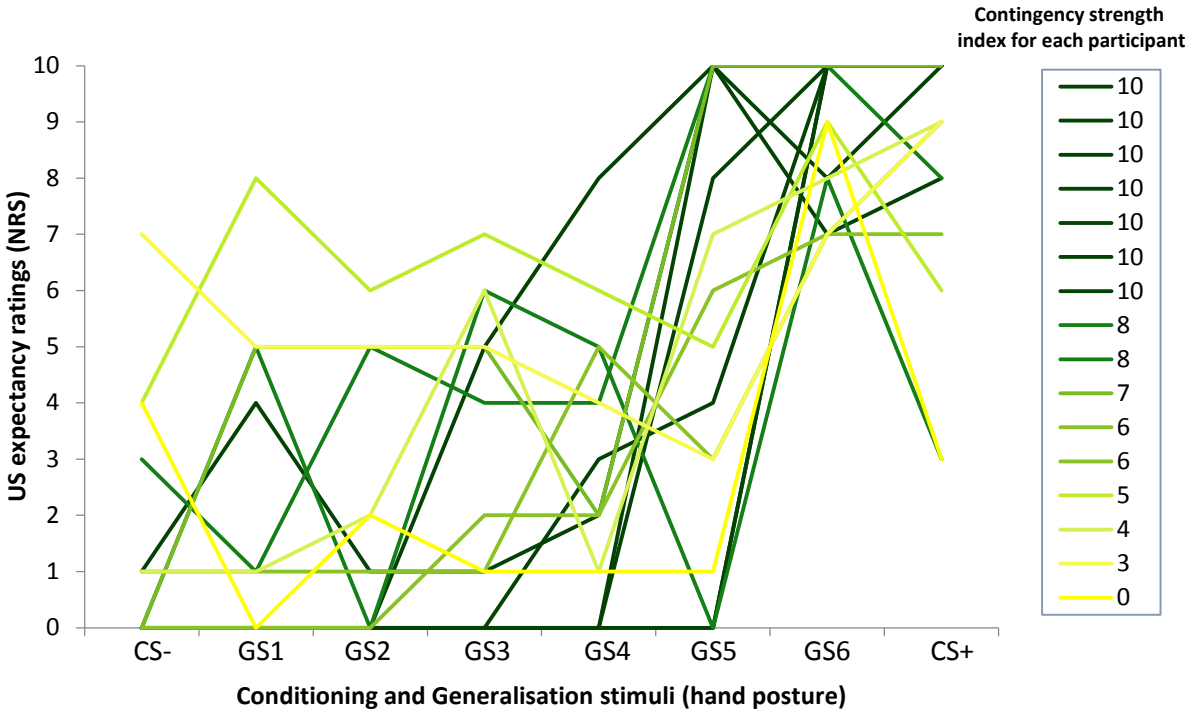
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Supplementary file A



Supplementary File A Figure. Individual participant US expectancy ratings for different hand postures.

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

6 Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

6. Are these faces different? Perceptual acuity for different faces – an electronic survey

Assessing whether participants can distinguish between similar stimuli is important in stimulus generalisation studies. This is because of the underlying assumption of stimulus generalisation: that the meaning associated with the CS+ by virtue of the US is extrapolated to stimuli that resemble the CS+. If, however, an individual fails to distinguish between the CS+ and the resembling stimulus, then this underlying assumption is incorrect and the interpretation of the results is limited.

Chapter 6 tests whether participants can distinguish between similar faces that belong to a particular set. This was done to ensure this the face set was suitable for the study in Chapter 7.

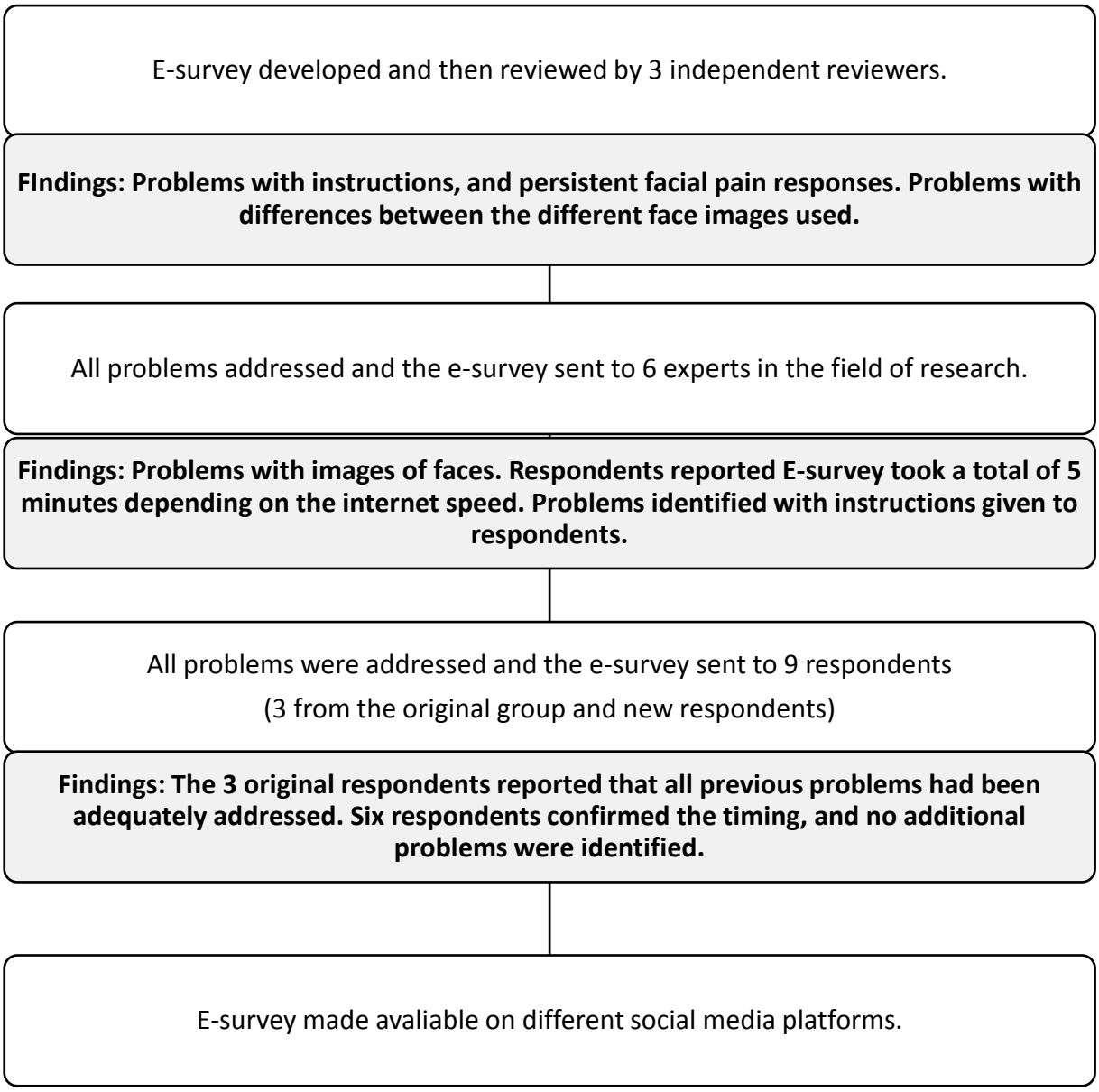


Figure 6.1. Piloting results for Chapter 6

Title: Are these faces different? Perceptual acuity for different faces – an electronic survey

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Abstract

Background: Recent studies of classical conditioning have used faces to interrogate pain-related responses. As the use of ecologically-valid stimuli in classical conditioning studies increases, so too does the importance of assessing perceptual discrimination between stimuli. This is further emphasised where classical conditioning studies investigate stimulus generalisation, because of the assumption made when interpreting the data: researchers assume that a conditioned response elicited by similar stimuli (generalisation stimuli) indicates that the participant has extrapolated the association between the original conditioned stimulus and unconditioned aversive stimulus to the novel, similar stimulus. However, if the participant has not perceived that the conditioned stimulus and the novel, similar stimulus are different, then this assumption is incorrect.

Methods: Given the complexity of faces and the need to assess distinguishability among stimuli in generalisation studies, we tested whether adults could distinguish between different faces, with a range of similarities, using an e-survey. The e-survey contained questions pertaining to demographics, current and past pain experience and persistent facial pain. The final section of the survey asked respondents to look at different face pairs and identify whether they perceived the two consecutive faces they had just viewed as the 'same' or 'different'.

Results: The majority of respondents could distinguish between the two distinct faces at the opposite ends of the spectrum. Furthermore, as the similarity between the different faces increased, the respondents' ability to accurately distinguish between them decreased.

Discussion: This study demonstrates the importance of assessing the distinguishability of similar stimuli, particularly if they are to be used to investigate stimulus generalisation.

Introduction

Perception and perceptual learning, the ability to discriminate between stimuli, has been demonstrated in different fields of research as of paramount importance (Matthen, 2015; Goldstone, 1998; Brattico, Nänen, & Tervaniemi, 2001; Pleger et al., 2006). This is because stimuli are made up of many different features on many different dimensions and the ability to distinguish between stimuli that are different in at least one dimension has been shown to reduce the experience of pain in certain persistent pain conditions (Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015). For example, Moseley, Zalucki, and Wiech (2008) showed that tactile discrimination training improved participants' ability to distinguish between the type and location of tactile stimuli, and decreased pain in participants with complex regional pain syndrome (CRPS). This finding has been corroborated with studies demonstrating that individuals with persistent pain report reduced pain levels after receiving treatments that improve their perceptual acuity of certain bodily sensations (Eads, Moseley, & Hillier, 2015; McNeil & Warrington, 1993; Stanton et al., 2013).

Face perception is one of the most developed visual skills in humans (Haxby, Hoffman, & Gobbini, 2000). The human ability to discriminate one face as uniquely different from another is unparalleled (Young & Ellis, 2013). This is thought to be due to the high social importance of recognising and distinguishing faces (Young & Ellis, 2013). Therefore, it is not surprising to find that facial stimuli are commonly used in different kinds of cognitive, neuroscience and psychological research (Haxby et al., 2000). Over the decades, faces have come to be used not only to investigate the processes involved in facial recognition and discrimination, but also as stimuli to modulate behavioural responses (Botvinick et al., 2005; Piekartz, Wallwork, Mohr, Butler, & Moseley, 2015; Suslow et al., 2013).

One body of work that has begun to incorporate face stimuli is that of classical conditioning studies. Recent research into conditioning-induced hyperalgesia (see Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015; Jensen et al., 2012, 2014 for further detail) has used emotionally neutral faces as conditioned stimuli. Classical conditioning has been used to demonstrate that an innocuous stimulus (e.g. an auditory tone) can elicit a conditioned response (e.g. fear) after it has been paired with an aversive stimulus (e.g. electrical shock; Lavond & Steinmetz, 2003). The concept that a tone can come to elicit a similar response to

that elicited by an electric shock—simply by virtue of an association between the tone and a shock—is a key component of classical conditioning (Miguez, Laborda, & Miller, 2014). Classical conditioning terminology often refers to the innocuous stimulus (e.g. the auditory tone) as the conditioned stimulus (CS), the aversive stimulus (e.g. the electrical shock) as the unconditioned stimulus (US), the natural response (e.g. fear) as the unconditioned response (UR) and the similar response as the conditioned response (CR; similar fear) (Lavond & Steinmetz, 2003). Stimulus generalisation, which is often investigated in classical conditioning studies, occurs when a particular response is elicited in the presence of stimuli that resemble the CS. In the context of protective responding, stimuli, which resemble the 'danger' stimulus ('danger' stimulus = CS; similar stimuli = generalisation stimuli or GSs), may also be perceived as dangerous because the knowledge about the CS-US relationship is extrapolated to the GS, such that the GS elicits, a similar protective response.

Some classical conditioning studies have recently moved away from the use of arbitrary conditioning stimuli and toward the use of more ecologically-valid ones (Domjan, 2005). As the use of ecologically-valid conditioned stimuli increases, so too does the importance of investigating processes such as perceptual discrimination (Zaman et al., 2015). Recent research has emphasised the particular importance of perceptual discrimination in stimulus generalisation because of the need to test the assumption that the GS is perceived as different from the CS. Vervliet and Van (2015) use the case of a participant showing a certain response to a CS and a similar response to a GS to argue that knowledge of the particiapnt's ability to distinguish between the CS and GS is critical for accurate interpretation of such a finding. If the participant has not discriminated between the CS and GS, then the response has not been elicited in the presence of a similar stimulus, but has in fact been elicited again in the presence of a perceptually identical stimulus. If the participant has distinguished between the CS and the GS, then the response can be said to have been 'generalised' from the CS to the GS. Consequently, failing to assess a participant's ability to distinguish between the CS and GSs carries the risk of drawning flawed inferences from the findings. In fact, the findings may not represent stimulus generalisation at all, but may actually represent the participant's ability to discriminate between the CS and GS (perceptual acuity).

Given the complexity of facial processing (Pitcher, Duchaine, & Walsh, 2014), the research regarding perceptual discrimination (Struyf, Zaman, Vervliet, & Van Diest, 2015) and its vital

role in stimulus generalisation (Vervliet & Van, 2015), this survey investigated respondents' ability to distinguish between different facial stimuli. This survey had three main aims: 1) to determine whether respondents can distinguish between two distinct faces; 2) to determine whether respondents can distinguish between faces that are various blends of two distinct faces. No other study that we are aware of has combined the features of two distinct faces to generate a range of 'blended' faces. Therefore, it was important to investigate whether the blended faces were identifiably distinct from each other. 3) Finally, this survey also investigated whether pain history variables (current or previous persistent pain, current or previous persistent facial pain) affected the respondents' ability to distinguish between two human faces. Given the evidence that persistent pain conditions (Zaman, De Peuter, Van Diest, Van den Bergh, & Vlaeyen, 2016; Zaman et al., 2015) and persistent facial pain (Forssell, Alstergren, Bakke, Bjørnland, & Jääskeäinen, 2016) are associated with differences in facial perception, we were particularly interested in the association between face pain and perceptual acuity. Given that some studies have shown age-related (Bidelman & Alain, 2015; Lacherez, Turner, Lester, Burns, & Wood, 2014) and gender-specific differences in perception (Thompson & Voyer, 2014), we also explored possible associations between differences in demographic variables (age and gender) and perceptual acuity.

Method

The 'face perception and pain' electronic survey used in this study was hosted by SurveyMonkey (www.surveymonkey.com; for full survey, see Attachment A). The methods and results are outlined below, and used the Checklist for Reporting Results of Internet E-Surveys (CHERRIES; Eysenbach, 2004).

Development and trials

The survey was developed by the principal researcher (KAT) and piloted on twelve volunteers. Volunteers were asked to provide feedback on the survey structure, and the clarity and phrasing of questions. The pilot volunteers provided the following feedback: first, the information outlining the survey needed clarification and synthesis; second, the responses to Questions eight and nine needed rephrasing, and third, errors in the skip logic needed to be fixed. Volunteers reported that the survey took approximately five minutes to complete, depending on the speed of their internet connection. The internet connection was

the greatest factor affecting time taken to complete the survey, because it determined the speed at which the images downloaded. The volunteers were blinded to the aims of the survey. They were asked to provide feedback regarding the face images. Volunteers identified that there were disparities in the borders and sizes of different face images. Following this feedback, all face images were edited to ensure that their borders and sizes were identical. Following final editing and ethical approval by the institution (protocol number: 0000033772), the survey was distributed online using various social media platforms (Facebook, Twitter and the Body in Mind webpage).

Outline of survey

The survey included questions about demographics, persistent pain experiences, persistent facial pain, and how the face images were perceived (i.e. whether consecutive images were the same or different). The first page covered information outlining the survey and consent. No remuneration or incentive was offered for responding to the survey. Participation was voluntary and any individual 18 years of age or over who had access to a computer with an internet connection could participate. The second page had two demographic questions (age and gender) and four persistent pain questions, with the last two pain-related questions focusing on persistent facial pain. The definitions for persistent pain and persistent facial pain descriptions used in this survey were adapted from The International Association for the Study of Pain (IASP) definitions (Merskey & Bogduk, 2016). The IASP facial pain diagnostic criteria were used as responses to Question nine (IASP, 2016). All questions were displayed in a standard order. Skip logic was used only on related questions where the respondent had answered 'no' to the initial question. All other questions were mandatory. The 'previous' button option was removed from the survey so that respondents could not switch between two faces multiple times to compare them before answering the question. Respondents were made aware of this and were encouraged to look at each face and then move on to the picture or question. It was also emphasised that the survey should take approximately five minutes, so as to discourage respondents from spending too long studying any of the faces.

Face discrimination task

A simple task was used to investigate the distinguishability of the face images (Goldstone, 1998). Respondents were asked to identify whether the two face images that they had just viewed were of the same face, or two different faces. This task allowed for perceptual acuity to be investigated, because an accuracy rate over 50% for any particular face pair is better than chance (Goldstone, de Leeuw, & Landy, 2015). It is important to note that, while perceptual acuity and perceptual discrimination are closely linked, they are subtly different. Perceptual acuity is an outcome of the process of perceptual discrimination; and, because perceptual discrimination can change over the course of time, so too can perceptual acuity (Zaman et al., 2015). The outcome assessed in this survey was perceptual acuity.

Generation of blended faces

The two distinct faces (face A and face I, see Figure 1) belonged to different individuals and were drawn from the Karolinska Directed Emotional Faces (KDEF) set (www.emotionlab.se/resources/kdef; Lundqvist, Flykt, & Öhman, 1998). The set consists of pictures of 70 individuals (35 female, 35 male; mean age: 25 years; age range 20—30) with seven different facial expressions per individual. A gradient of faces was created by blending different ratios of the initial two distinct faces (face A and face I) to generate a total of seven blended faces (face B to face H, Figure 1).

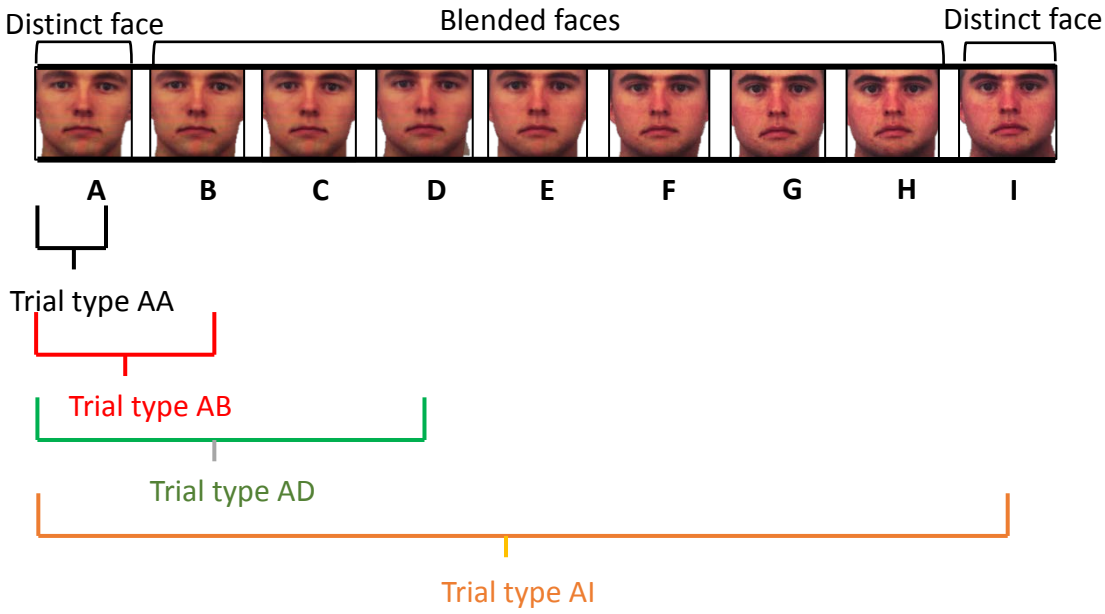


Figure 1. Similarity spectrum of faces. The distinct faces of two different people (face A and face I) are shown either side of a number of other face images, that were created by blending features of faces A and I to different degrees.

Trial type

In order to interrogate whether the distinct and blended face images were distinguishable, this survey tested four different combinations (i.e. face pairs) of the faces from the spectrum (Figure 1). Trial type AA could be one of four possible pairs of the same blended or distinct face images (e.g. face D and face D). Trial type AB could be one of four possible pairs of either the distinct or blended face images that were next to each other on the spectrum (e.g. face E and face F). Trial type AD could be one of four possible pairs of either blended or distinct face images that were two face images away from each other on the spectrum (e.g. face E and face H). Trial type AI consisted of the two distinct faces on the ends of the spectrum (face A and face I). The four face pairs in Trial type AA were used as oddball trials to control for a base rate of error in presenting sequential faces.

Randomisation of face pairs

The survey showed a total of sixteen pairs of faces (four blocks of four face pairs). The faces in a pair appeared consecutively, and a question followed asking whether the two faces just viewed were the same or different. Each face image was paired with another and allocated to a block, depending on the pairing, e.g. face B paired with face B was allocated to block type AA. Microsoft Excel was used to randomise the order in which the face pairs appeared. For example, a randomised order of Trial types might run: AA, AI, AA, AD, AB, AD, AI, AA, AI, AA. This process would continue until a block of sixteen trials had been randomised.

Data processing

If responses to the face pair questions were incomplete, the data for that participant's survey was excluded. Limited measures were undertaken to tidy the data. All responses regarding the face pairs were re-coded with either a 0 (for an incorrect response) or a 1 (for a correct response).

For each Trial type, the number of correct responses for each face pair was tallied and converted to a percentage. The respondents' response accuracy was used as an indication of perceptual acuity. For example, one respondent had correct responses for all four of the face pairs in Trial type AD, but no correct responses for Trial type AB. Therefore, this

respondent had 100% response accuracy for Trial type AD and 0% response accuracy for Trial type AB. The respondents were then re-grouped depending on whether they had experienced persistent pain (previous, any location), still experienced persistent pain (current, any location), experienced persistent *facial* pain (current), as well as by age and gender. Analysis of whether these pain history and demographic variables were associated with a decrease in respondent's response accuracy was conducted.

Results

The survey link was made available on August 9th 2016 through social media and was accessible for four weeks. The survey was accessed 99 times. Of the 99 collected surveys, two respondents did not consent to participating in the survey and therefore did not move past the first page. Nine respondents' survey responses were incomplete, so their data was excluded, leaving a total of 88 complete surveys for analysis.

The results are presented in three ways: first, the results from the full sample are reported, to show the overall demographics of the survey sample. Second, the results are grouped according to respondents' perceptual acuity for Trial types AA, AB, AD and AI. Third, the results for each Trial type were grouped according to the response to each pain history (current or previous persistent pain, current or previous persistent facial pain) and demographic variables (age and gender). This allowed analysis of whether any variables were associated with differences in the ability to distinguish between face pairs or, in the case of Trial type AA, to identify a face pair as the same.

Results from the full sample

Of the 88 respondents who completed the survey, 77% were female, with 30-39 year olds being the most highly represented (34%). Of the 53% of respondents who had experienced persistent pain, 57% reported still experiencing persistent pain. The 60 years and older age group had the highest percentage of people who had experienced persistent pain. However, with the exception of the 21-29-year age group, at least 50% of people from each age range group had experienced persistent pain. Fifty-seven percent of females reported having experienced persistent pain, compared to only 35% of males. Eight percent of respondents reported that they had experienced persistent facial pain. Of this 8%, only 3% no longer experienced persistent facial pain. Amongst the respondents who reported having

experienced persistent facial pain, temporomandibular joint (TMJ) disorders were the most common complaint (60% of this group).

Perceptual acuity for each Trial type

Response accuracy data for each Trial type were analysed and are outlined in Figure 2 (see also Appendix B). The Trial type with the highest number of respondents with 100% accuracy was Trial type AI (*n* = 83, 94%). The next best performance was recorded for Trial type AA, with 72 (82%) respondents achieving 100% accuracy, followed by Trial type AD with 16 (18%) respondents with 100% accuracy. No respondent achieved 100% accuracy for Trial type AB. In fact, 51 respondents (58%) failed at every Trial type AB presented.

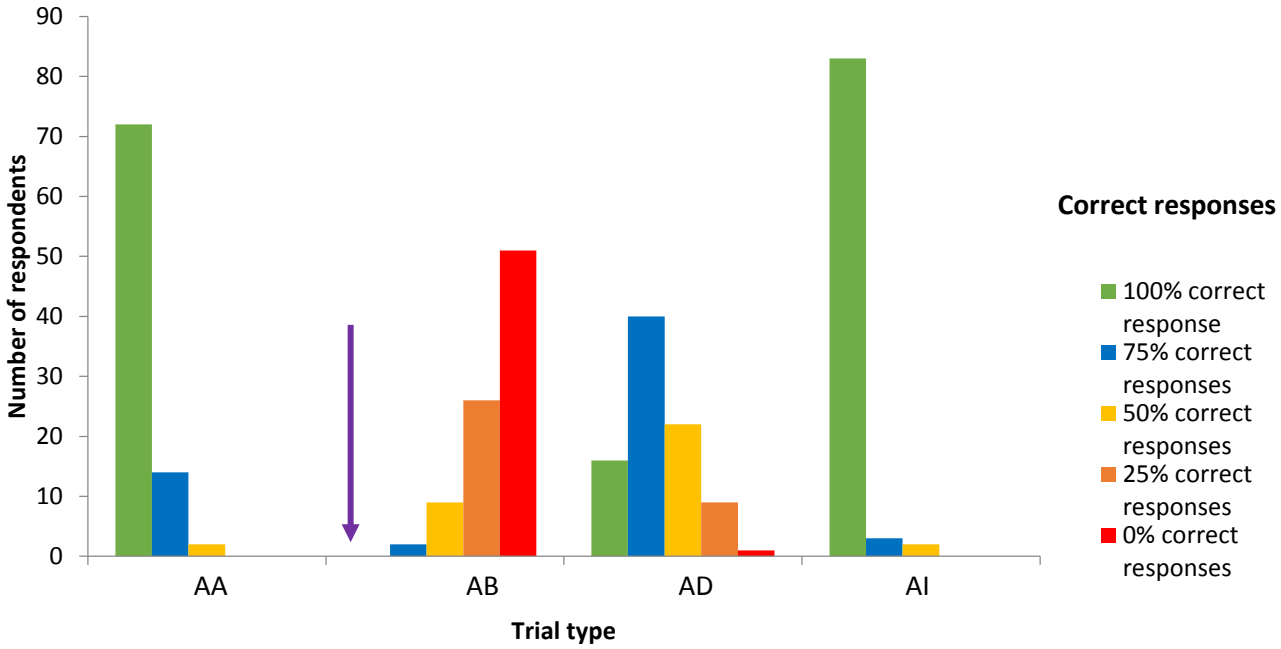


Figure 2. Response accuracy for face distinction trials. Note the high error rate for Trial type AB: No respondents were 100% correct (purple arrow), and 51 respondents (red bar) were 0% correct.

Respondents' response accuracy for each Trial type was then re-grouped according to their reported pain histories (current or previous persistent pain, current or previous persistent facial pain) and demographic variables (age and gender; Figs. 3-6).

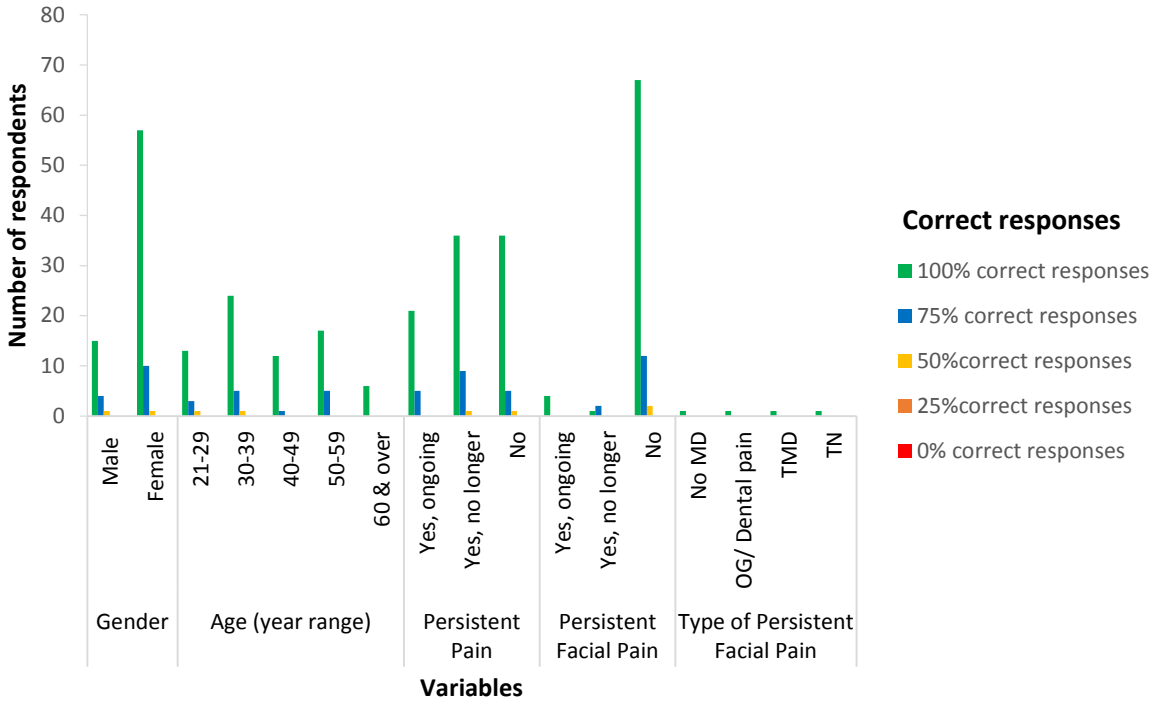


Figure 3. Response accuracy for Trial type AA for each pain history and demographic variable. No MD: No medical diagnosis, OG/dental pain: Odontogenic/ dental pain, TMD: Temporomandibular disorder, TN: Trigeminal neuralgia.

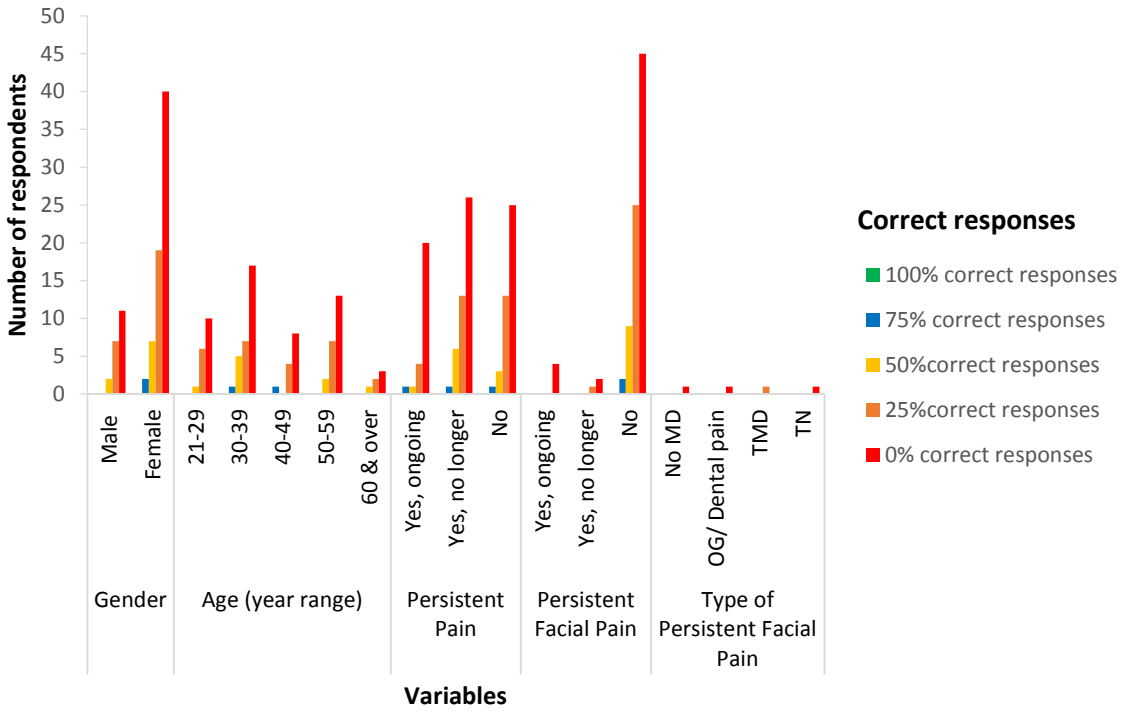


Figure 4. Response accuracy for Trial type AB for each pain history and demographic variable. No MD: No medical diagnosis, OG/dental pain: Odontogenic/ dental pain, TMD: Temporomandibular disorder, TN: Trigeminal neuralgia.

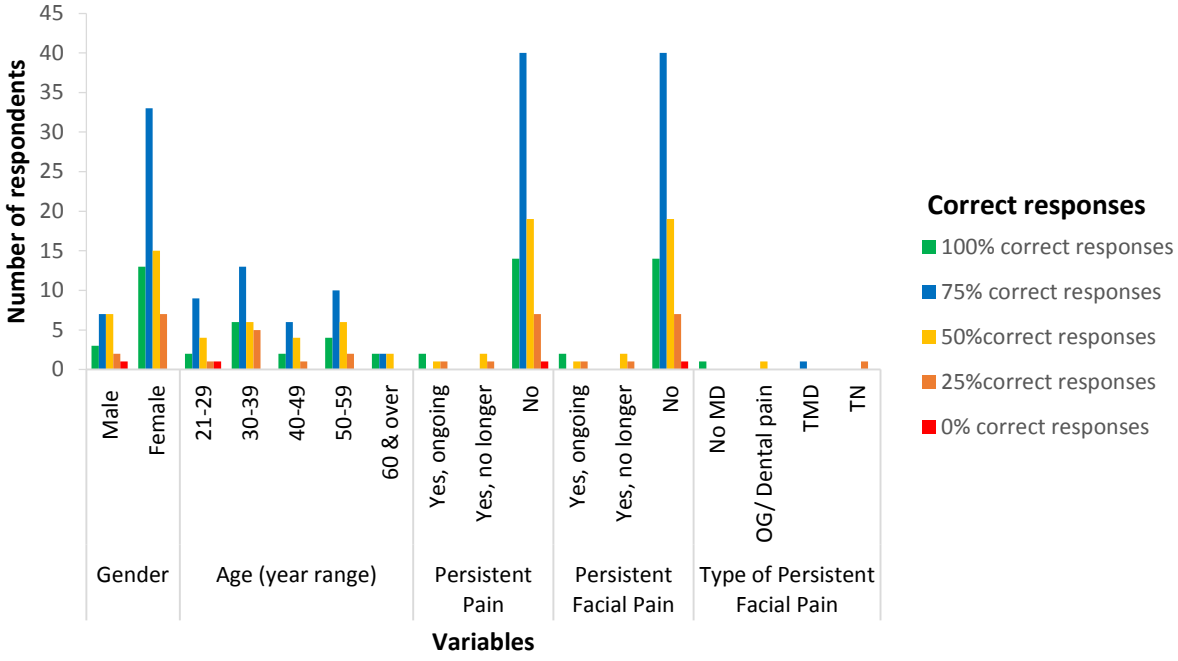


Figure 5. Response accuracy for Trial type AD for each pain history and demographic variable. No MD: No medical diagnosis, OG/dental pain: Odontogenic/ dental pain, TMD: Temporomandibular disorder, TN: Trigeminal neuralgia.

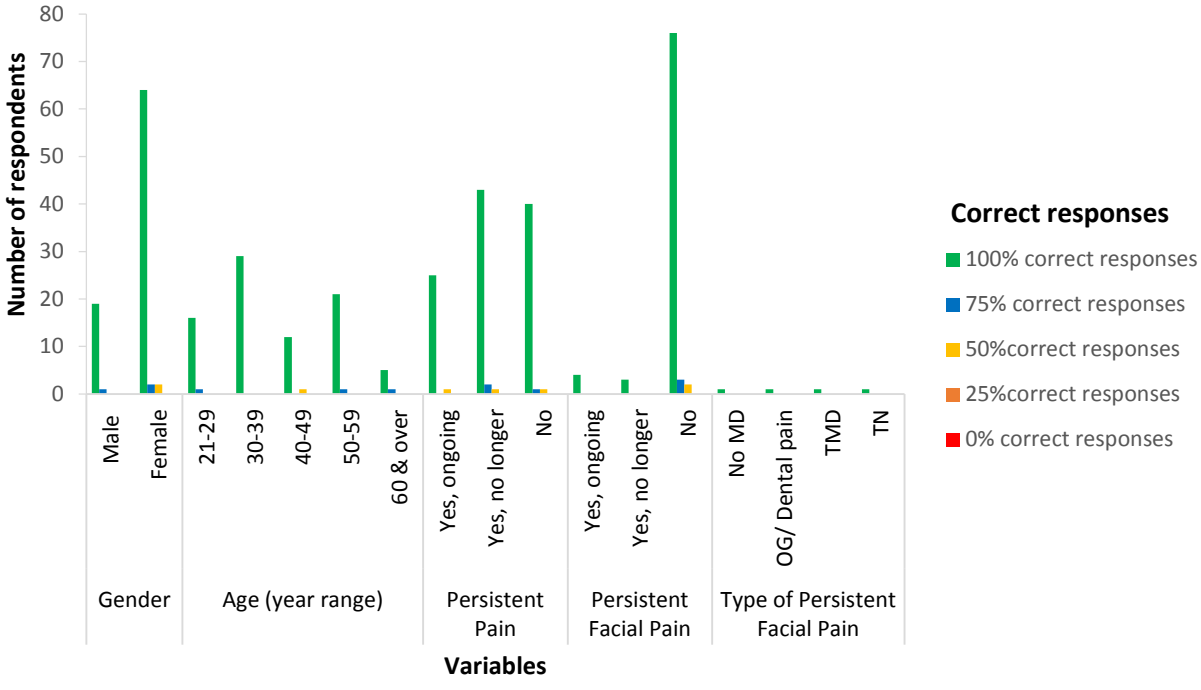


Figure 6. Response accuracy for Trial type AI for each pain history and demographic variable. No MD: No medical diagnosis, OG/dental pain: Odontogenic/ dental pain, TMD: Temporomandibular disorder, TN: Trigeminal neuralgia.

Discussion

This survey had three aims. The first aim was to establish whether respondents could distinguish between two distinct faces. The majority (94%) of respondents distinguished the two distinct faces with 100% accuracy (Trial type AI). This was an encouraging result, because it meant that these two faces were sufficiently distinct to be used as conditioning stimuli in a differential classical conditioning study. Furthermore, individual differences in the pain history and demographic variables measured (current or previous persistent pain, current or previous persistent facial pain, age and gender) had no discernible association with respondents' ability to distinguish between face A and face I. The second aim was to establish whether respondents could distinguish between faces that were differently balanced blends of the original two distinct faces. Mixed results were found for the respondents' ability to distinguish between the blended faces in the different Trial types. The majority (82%) of respondents were able to identify that the blended faces in Trial type AA (same faces) were identical. This was an important result, because it highlighted that the baseline error for sequentially-displayed face images was reasonably low. The results for the other two trial types were not as promising. None of our 88 respondents had 100% accuracy for Trial type AB, and only 16 of 88 respondents had 100% accuracy for Trial type AD. Finally, the third aim was to determine whether pain history variables (current or previous persistent pain, current or previous persistent facial pain) affected respondents' ability to distinguish between two human faces. The variables of gender, age, persistent pain, persistent facial pain and type of persistent facial pain, were not associated with differences in accuracy. The majority of respondents who reported persistent pain had 100% accuracy for Trial type AA and Trial type AI, 75% accuracy for Trial type AD, and 0% accuracy for Trial type AB – similar results to those who did not report persistent pain.

In the current study, the more closely the blended faces resembled each other, the harder they were to distinguish. This is to be expected, and indicates that the blending procedure successfully generated a series of face stimuli that formed a continuum of similarity between face A and face I. However, the poor performance on AB Trials indicates that adjacent faces on this continuum were too similar for use in studies that rely on perceptual discriminability between adjacent stimuli, such as studies of stimulus generalisation. The most obvious explanation for this lack of distinguishability is that the differences between adjacent stimuli were too subtle to allow for easy discrimination. Using fewer faces on the same continuum

could produce stimuli that are less similar and easier to distinguish. Another potential explanation considers categorical perception—the perception of the world according to categories (Goldstone & Hendrickson, 2010). For example, when looking at a rainbow, most individuals see seven different colours, despite the rainbow comprising the full spectrum of visible light. That is, the perception of a rainbow is categorised into the seven distinct colours despite the fact that there are more subtle colour variations available (e.g. turquoise, aqua; Goldstone & Hendrickson, 2010). What this example demonstrates is that perception is moulded so that individuals are better able to distinguish between stimuli that fall into distinct categories rather than when the stimuli are subtle variations of the same category (Goldstone, 1994; Goldstone & Hendrickson, 2010). Therefore, similar to the way that some individuals have difficulty in distinguishing different shades of blue, the respondents may have had difficulty distinguishing between similar blended faces. Although this explanation rests on a different perceptual process, its consequences for the application of these stimuli in the experimental context are unchanged: generating fewer stimuli for the spectrum would likely facilitate better discrimination. However, a third explanation for the poor performance could be that the faces were not paired with a motivating stimulus. Some studies have found that participants' ability to distinguish between very similar stimuli can be improved by pairing the stimuli with an aversive stimulus (Zaman et al., 2015). For example, Li, Howard, Parrish, & Gottfried (2008) used odour enantiomers – pairs of odours that smell identical because they are molecular mirror images of each other — to investigate odour discrimination. One pair of odours was used in a fear conditioning task, such that one of the enantiomers was paired with an aversive stimulus (becoming a threat cue) and the other was not (becoming a safety cue). Li et al. (2008) found that participants were better able to distinguish between odours that had been used in the fear conditioning procedure than between odours that had not been used in the fear conditioning procedure (control enantiomers). Therefore, providing motivation for learning could have improved respondents' performance.

The current results have important implications for the design of future studies. First, they emphasise the importance of investigating perceptual discrimination. This is of particular relevance in studies of learning mechanisms such as conditioning and generalisation (Vervliet & Van, 2015). A common assumption made when interpreting the results of generalisation studies is that the CS is actually perceived to be different from the GS (Struyf

et al., 2015), but this is not necessarily true. In fact, the results could be attributed to either stimulus generalisation or perceptual acuity. In such studies, failing to assess perceptual discrimination would preclude the clear interpretation of results.

When interpreting the current study's results, certain limitations should be considered. First, the simple task of distinguishing between the face pairs required memory (Berry et al., 2010). The two faces were displayed consecutively and, therefore, the respondents were required to remember the first face in order to compare it to the second face. The use of consecutive order was appropriate, because the order mimicked the way in which such stimuli are presented in most experimental research. However, we did not assess respondents' memory performance, and perceptual discrimination between the very similar faces could have been limited by their ability to remember the subtle differences between the two faces (Berry et al., 2010). Second, the task required attention (Arathorn, 2005). It is possible that, when viewing the faces, the respondents may have attended to some features but ignored others (Chubb, 2005; Deco & Rolls, 2005). If respondents ignored certain features of the face, and these were the features that differed, then this too would have influenced performance (Martinez-Trujillo & Treue, 2005). We were unable to control for contextual, instructional and motivational variables that may have influenced which facial features attracted participants' attention (Palermo & Rhodes, 2007). Third, we did not assess if respondents had vision problems or, if they did have vision problems, whether their vision had been corrected (e.g. with glasses). This failure to assess visual acuity means that if respondents were unable to see the faces, their poor results would be due to poor vision, rather than poor perceptual acuity. However, it seems reasonable to assume that most participants would wear the necessary visual assistive devices to allow them to adequately visualise the computer screen on which they completed the survey. Finally, we did not assess for co-morbidities such as prosopagnosia (McNeil & Warrington, 1993; cognitive disorder of face perception) or schizophrenia (Onitsuka et al., 2006) that may have influenced face perception or recognition and, therefore, the ability to distinguish between faces.

Conclusion

This survey investigated three questions about the perceptual discrimination of human faces. The first question was whether the faces of two different people were distinct to participants. The majority of respondents were able to correctly distinguish between the two faces. The second question was whether faces on a 9-step continuum of faces, made by gradually blending the two distinct faces, were distinguishable. Mixed results were found for this, with the majority of respondents unable to distinguish between blended faces that were adjacent (one step apart) on the continuum. Finally, the third question was to identify whether pain history or demographic variables were associated with a difference in perceptual acuity. No major associations between perceptual acuity and the different variables were identified. Future studies should assess participants' ability to discriminate between experimental stimuli. There is also a need to determine the roles that higher-order cognitive functions, such as memory and attention, may play in perceptual acuity tasks.

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Attachment A

Perception of Faces Survey

Information about this survey

1. Please take a moment to read the below information about this survey:

The Body in Mind Research Group at the University of South Australia invites you to be part of a survey investigating perception and faces. This survey will collect data regarding different faces and general pain information. There are 7 questions about your age, gender and pain experience and all questions are mandatory and require a response. The mandatory information required relates to your consent to participate in this survey, age and gender, previous and current pain experience and about the faces.

The survey will take approximately 5 minutes to complete. You will be shown faces in pairs. In each pair, Face 1 will be shown immediately before Face 2. You will be asked to decide whether the two faces you have just seen are the same or different. Write your answer in the response box that will appear on a new page after each pair of faces. You will not be able to go back to view the face pair again. There are 16 pairs of faces (or 32 individual faces) and each face is shown on a new page.

We want to find out how easily people can distinguish between faces that are similar. All the images shown in this survey represent a fixed time point in time. We will not show you differently timed pictures of the same face. We will show you the same face twice or we will show you two different faces. Thank You.

- ◯ I have read the above statement and want to participate
- ◯ I have read the above statement and do not want to participate



Perception of Faces Survey

Consent to participate in survey

2. Please read the below information carefully:

Participants aged 18 years and older are invited to take part.All data collected from you will be stored securely for 5 years within the Body in Mind research center at University of South Australia, City East. The researcher will take every care to remove responses from any identifying material as early as possible. Your participation is voluntary. Likewise individuals' responses will be kept confidential by the researcher and participants will not be identified in the reporting of the research.
However the researcher cannot guarantee the confidentiality or anonymity of material transferred by email or the internet.
A copy of the final research report will not be directly emailed to you but if you wish to access the report, they will be available on a publicly viewable website- 'bodyinmind.org'. All de-identified information will be used only for research purposes.

I have read the Participant Information Sheet and understand the nature and purpose of the research project
I understand the purpose of the survey and my involvement in it.
I understand that I may withdraw from the survey at any point.
I understand that while information gained during the study may be published, I will not be identified and my personal results will remain confidential and de-identified.
I understand that the data obtained during the study will be coded and de-identified.
I understand that the de-identified data will be used for research.

This project has been approved by the University of South Australia's Human Research Ethics Committee. If you have any ethical concerns about the project or questions about your rights as a participant please contact the Executive Officer of this Committee at +61 8 8302 3118 or via email vicki.allen@unisa.edu.au.

Primary researcher's contact details: Kerwin Talbot Phone: (08) 83021295 Email: Kerwin.Talbot@mymail.unisa.edu.au

By ticking the 'I agree' box you are consenting that you are 18 years or older and you consent to the responses you provide being used in research.

- ◯ I agree
- ◯ I disagree



Perception of Faces Survey

Demographics and Pain

3. What is your age?

- 18-20
- 21-29
- 30-39
- 40-49
- 50-59
- 60 or older

4. Are you male or female?

- Male
- Female

5. Have you ever experienced persistent pain (For the purpose of this survey persistent pain is described as pain on most days for longer than 3 months)

- Yes
- No

6. Do you currently have persistent pain? (For the purposes of this survey persistent pain is described as pain on most days for longer than 3 months)

- Yes
- No



Perception of Faces Survey

Pain Info

7. How much does your persistent pain limit you?

- ◯ It does not limit me at all
- ◯ It limits me a little bit
- ◯ It limits me moderately
- ◯ It limits me substantially
- ◯ It limits me most of the time

8. Have you ever suffered from persistent facial pain? (For the purpose of this survey, persistent facial pain is described as pain in the mouth, jaws and face on most days for longer than 3 months)

- ◯ Yes, I still experience persistent facial pain
- ◯ Yes, but I no longer experience persistent facial pain
- ◯ No

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Perception of Faces Survey

Persistent Facial pain

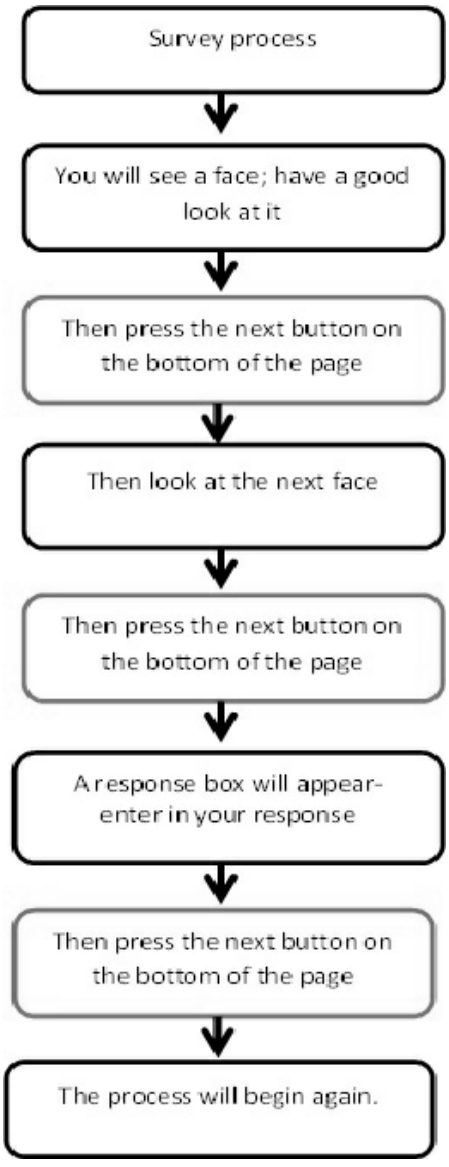
9. What type of persistent facial pain have you been diagnosed with?

- ◯ Orofacial pain
- ◯ Neurovascular Orofacial pain
- ◯ Persistent Dento-Alveolar Pain Disorder
- ◯ Odontogenic/Dental Pain
- ◯ Burning Mouth Syndrome
- ◯ Temporomandibular Disorders
- ◯ Persistent Idiopathic Facial Pain
- ◯ Trigeminal Neuralgia
- ◯ Glossopharyngeal Neuralgia
- ◯ I have not been medically diagnosed

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Perception of Faces Survey

Survey process



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Perception of Faces Survey

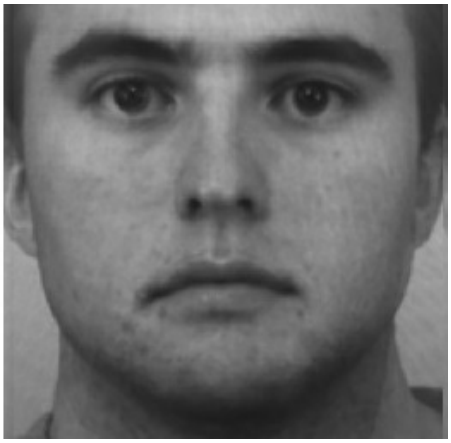
Face 1



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Perception of Faces Survey

Face 2



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Perception of Faces Survey

Question

10. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

11. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

12. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2



UniSA Sansom Institute for Health Research

Perception of Faces Survey

Question

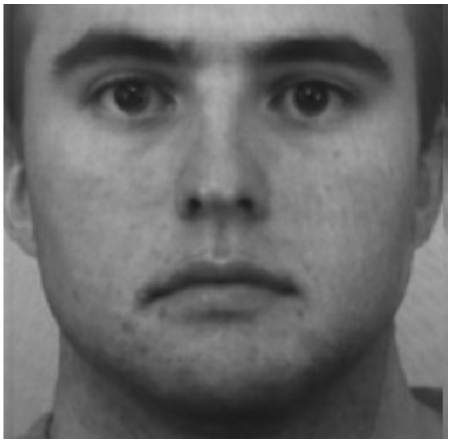
13. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2



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Perception of Faces Survey

Question

14. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



Face 2



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Perception of Faces Survey

Question

15. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2



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Perception of Faces Survey

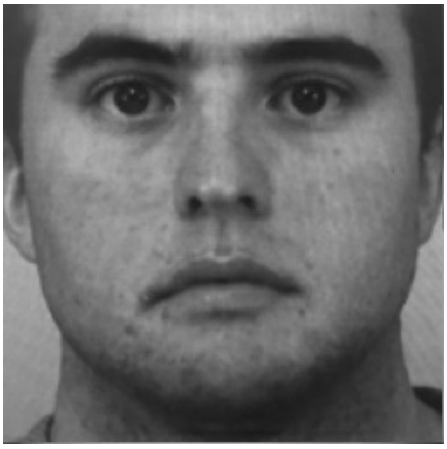
16. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



Face 2





Perception of Faces Survey

Question

17. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2



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Perception of Faces Survey

Question

18. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

19. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

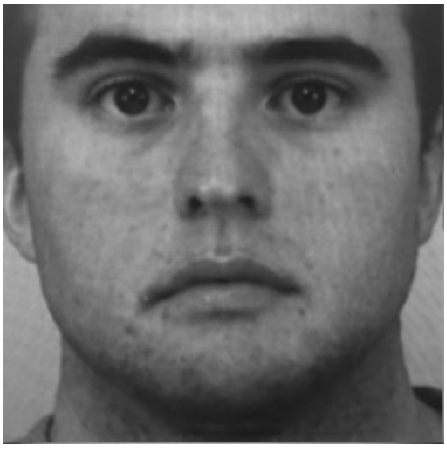
20. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

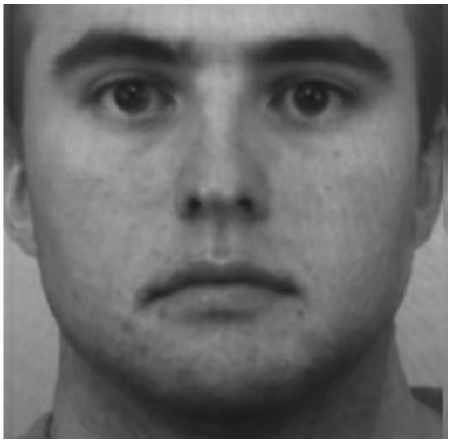
21. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

UniSA Sansom Institute for Health Research

Perception of Faces Survey

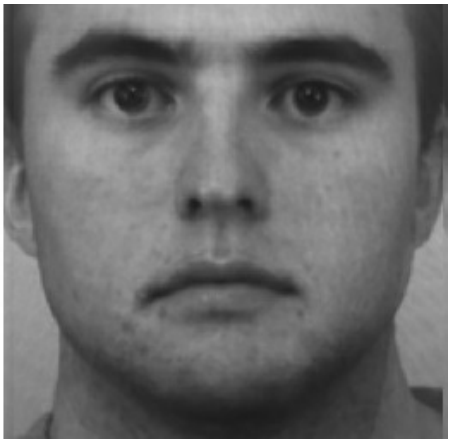
Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

22. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

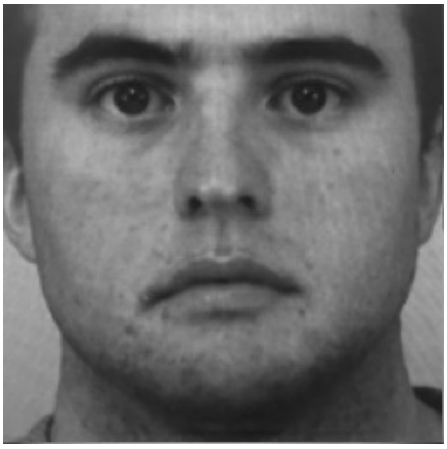
Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

23. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

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Perception of Faces Survey

Face 1



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Perception of Faces Survey

Face 2





Perception of Faces Survey

Question

24. Are the two consecutive faces you have just seen the same OR different?

- ◯ Same
- ◯ Different

Perception of Faces Survey

We really appreciate your participation in this survey- you are really helping us to better understanding facial pain and perception.
Thank you very much for your participation and helping us with this survey.

Attachment B

Table 1. Percentage of correct responses for the 16 face pairs

	Trial type AA	Trial type AB	Trial type AD	Trial type AI
	Correct responses			
	(%)	(%)	(%)	(%)
Mean	93	14	68	98
Face pair 1	99	17	30	98
Face pair 2	97	28	83	98
Face pair 3	90	7	64	99
Face pair 4	86	5	93	98

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

7 Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

7. Healthy controls show conditioned generalisation of hyperalgesia to face images.

Chapter 5 highlighted the possibility that the choice of CS, US, the rating scale, and the robustness of the each, are critical determinants in the success of studies investigating classical conditioning and generalisation effects. Given these findings, the choice of each was re-evaluated and the CS, US and rating scale were changed in Chapter 7. The imprecision hypothesis of chronic pain assumption of the role of generalisation within persistent pain conditioned was investigated again.

Chapter 6 highlighted that respondents' ability to distinguish between faces decreased as their similarity increased. Therefore, the faces were further piloted and refined before they were used as conditioned and generalisation stimuli for the study described in this chapter.

Several different step-wise progressions (mA) and inter-pulse intervals(sec) of the electrical stimuluation were trialled on 6 participants

Findings:

Electrical stimulus intensity (mA)	Step-wise progression (mA)	Inter-pulse interval (sec)
Start: 2.0	1.0	50-60
Once 9.0 reached	1.5	50-60
Once painful (intensity variable)	3.0	50-60

Different types of electrodes were tested on 6 participants to determine the best way to deliver the three intensities (high, medium and low) of the stimulus.

Findings: Transcutaneous electrodes were selected to deliver the low intensity electrocutaneous stimuli. Intracutaneous electrodes were selected to deliver the medium and high intensity electrocutaneous stimuli.

Entire experiment programmed and trialled on 5 participants

Findings:
I. **Trial flow timing for each experimental block was appropriate**.
II. **The CS and GS were displayed the correct number of times.**
III. **The written instructions were unclear**
IV. **Some problems with ease of rating**
V. **Average time taken to rate was approximately 3000 ms**
VI. **Timing and stimulus types were correct**.
VII. **The limits set on the button option programmed for this experiment were accurate**
VIII. **The output file where all the participants' responses were programmed to be recorded had a timing problem (the times were recorded incorrectly).**
IX. **The entire experiment took an average of 90 minutes**

Entire experiment re-piloted on 3 participants

Findings
III. **Written instructions were clear.**
IV. **Ease of rating was improved.**
VIII. **Recording of timing in output file was fixed.**

Figure 7.1. Piloting results for the experimental procedure used in Chapter 7

Entire experiment programmed and piloted on 5 participants.
The US expectancy ratings and pain intensity ratings for the different faces proposed to be used as the CS and GS in Chapter 7 were assessed.

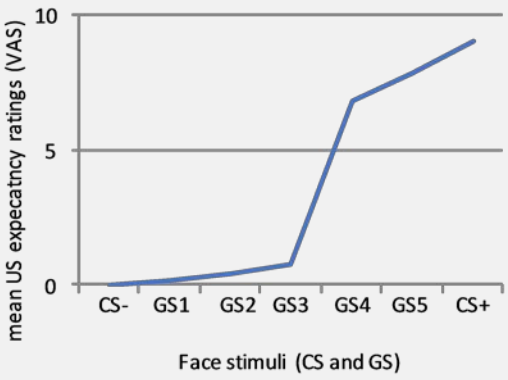


Figure P1. Piloting results of US expectancy ratings for the CS and five GS.

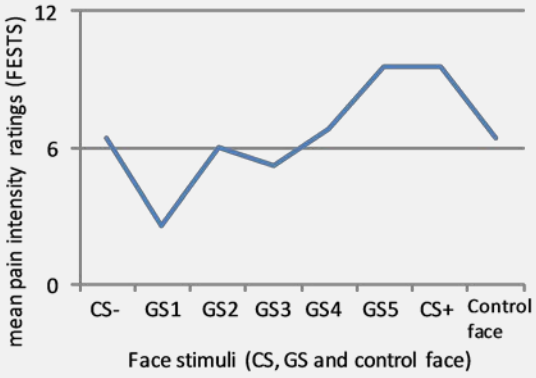


Figure P2. Piloting results of pain intensity ratings for the CS and five GS.

Face stimuli for GS1 and GS2 US expectancy and pain intensity were rated similarly. Therefore, they were deemed to be indistinguishable from each other and were removed.

Entire experiment re-piloted on 5 participants.
The US expectancy ratings and pain intensity ratings for the different faces proposed to be used as the CS and GS in Chapter 7 were assessed.

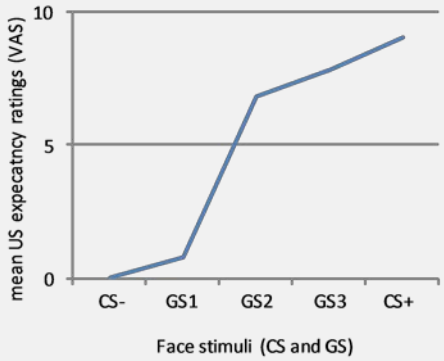


Figure P3. Piloting results of US expectancy ratings for the CS and three GS.

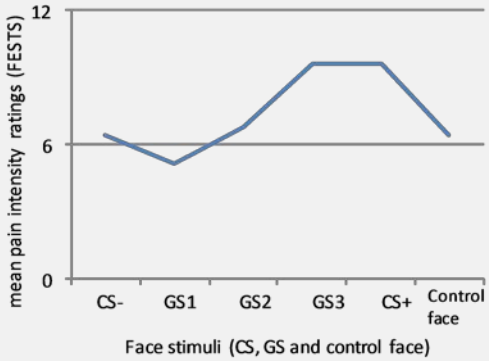


Figure P4. Piloting results of pain intensity ratings for the CS and three GS.

Ratings of US expectancy and pain intensity were different. The remaining face stimuli were used in Chapter 7.

Figure 7.2. Piloting results for the GS and CS used in Chapter 7

Title: Healthy controls show conditioned generalisation of hyperalgesia to face images

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Conflict of interest: GLM has received support from: Pfizer Australia, Kaiser Permanente, Results Physiotherapy, Agile Physiotherapy, Workers' Compensation Boards in Australia, Europe and North America, the International Olympic Committee and Port Adelaide Football Club. GLM and VJM receive speaker fees for lectures on pain and rehabilitation. GLM receives royalties for books on pain and rehabilitation. All the other authors declare no conflicts of interest.

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Abstract

After decades of research into pain, the factors that determine whether an acute pain episode becomes chronic remain unknown. Recent research into associative learning has highlighted the potential for hyperalgesia to be a conditioned response. The current study investigated whether conditioning-induced hyperalgesia generalises to similar but novel stimuli. To do this, a differential classical conditioning paradigm was used, where one face stimulus (CS+) was paired with an unconditioned, highly painful, electrical stimulus; and another face stimulus (CS-) was paired with a non-painful, electrical stimulus. Following acquisition, participants received a moderately painful stimulus paired with not just the CS+ and CS-, but also three novel but similar face images (generalisation stimuli). This study found that participants rated the moderately painful stimulus more painful if it was preceded by the CS+ than if it was preceded by the CS-. Moreover, conditioning-induced hyperalgesia decreased as the similarity of the novel faces to the CS+ decreased. This study confirms other studies' findings on conditioning-induced hyperalgesia, and extends them to show that conditioning-induced hyperalgesia can be generalised to similar but novel stimuli. These results suggest that associative learning may play a role in the experience of pain.

Introduction

The factors that determine whether an acute pain episode progresses to become chronic pain remains unknown. Over the past two decades, pain research has improved (Moseley & Butler, 2015). Such improvements have ranged from gaining a better understanding of the complexities of the nociceptive system, to the psychological mechanisms which modulate pain (Moseley & Arntz, 2007; Wiech, Ploner, & Tracey, 2008; Wiech & Tracey, 2009; Woolf, 2009). For example, the Fear-Avoidance Model (FAM) outlines how associations, such as a negative appraisal of pain, can lead to pain-related fear, which can drive avoidant behaviours and promote disuse and disability (Vlaeyen, Crombez, & Linton, 2016).

Recent proposals (Moseley & Vlaeyen, 2015; Vlaeyen, 2015) and experiments (Harvie, Meulders, Madden, et al., 2016; Harvie et al., 2015; Madden et al., 2016) have reignited the possibility that associative learning influences the transition to chronic pain. For example, a study by Harvie et al. (2015) showed how vision can influence the reported onset of pain during neck rotation in people with chronic neck pain. Other studies have shown that cues which are associated with threat are more likely to be rated as painful (Arntz & Claassens, 2004; Moseley & Arntz, 2007, Wiech et al., 2010). This evidence has driven the idea that non-nociceptive stimuli can perhaps become associated with a painful event and subsequently modulate a pain response. For example, one study, using a differential classical conditioning paradigm, paired one vibrotactile conditioned stimulus (CS+) with a painful laser stimulus (US) and another vibrotactile conditioned stimulus (CS-) with a non-painful laser stimulus (US; Madden et al., 2016). Following acquisition, at-pain-threshold laser stimuli were delivered and were paired with either the CS+ or the CS-. This study showed that a greater percentage of at-pain-threshold laser stimuli were rated as painful when paired with the CS+ than when paired with the CS-.

A recent proposal, the imprecision hypothesis of chronic pain (Moseley & Vlaeyen, 2015), posits that the generalisation of conditioning-induced hyperalgesia might explain the clinical phenomenon whereby an already painful stimulus, and other similar stimuli, are reported to be more painful. That is, if an individual was to burn themselves, then after this experience they might report a heat stimulus that previously was only moderately painful to be more

painful, because of an association between heat and danger (Vlaeyen, 2015). Moreover, other heat stimuli that are similar to the moderately painful heat stimulus may also be reported as more painful after the experience of being burned (Vlaeyen, 2015). That is, hyperalgesia has been generalised because the meaning of danger has been extrapolated to similar stimuli. This may help an individual to predict potential dangers and protect themselves from them.

Classical conditioning studies have demonstrated that an already painful stimulus can become more painful due to a conditioned effect (i.e. conditioning-induced hyperalgesia). Several studies have used a differential classical conditioning paradigm where one face image (CS+) was paired with a highly painful thermal stimulus, and another face image (CS-) was paired with a non-painful thermal stimulus (Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015; Jensen et al., 2012, 2014). During testing, a moderately painful thermal stimulus was paired with either the CS+ or the CS-. These studies found that a moderately painful heat stimulus was reported as more painful when paired with the CS+ than when paired with the CS-. That is, a painful stimulus was reported as being more painful when presented with the face image previously associated with the highly painful thermal stimulus.

Whether or not conditioning-induced hyperalgesia generalises to similar stimuli has not been established. One study we are aware of explored whether conditioning-induced hyperalgesia generalised to similar vibrotactile stimuli (Harvie et al., 2016). However, this study did not demonstrate generalisation of conditioning-induced hyperalgesia. However, a limitation of this study was that the conditioned generalisation of hyperalgesia was tested after a non-reinforced Generalisation phase. Therefore, it is plausible that any generalisation effect may have already been extinguished, resulting in non-significant findings (Lavond & Steinmetz, 2003). Given that the evidence for the generalisation of conditioning-induced hyperalgesia is limited, this current study investigated whether it is possible.

We hypothesised that a painful stimulus would be rated as more painful if it was preceded by a CS+ rather than a CS- (i.e. demonstrating conditioned-induced hyperalgesia). Moreover, we hypothesised that the magnitude of conditioning-induced hyperalgesia would decrease as the similarity of the novel face stimuli to the CS+ decreased. Our secondary aims were to

investigate whether a difference in fear-avoidance beliefs, and catastrophising, was associated with a difference in the extent of generalisation of conditioning-induced hyperalgesia. This was investigated for two main reasons. First, the fear-avoidance beliefs (FAB) that an individual has regarding pain will affect how they assess and respond to a painful experience (George, Fritz, & McNeil, 2006; Waddell, Newton, Henderson, Somerville, & Main, 1993). Second, whether catastrophising can influence the generalisation of conditioning-induced hyperalgesia in healthy controls has received little attention. That is, we do not know whether an exaggerated negative appraisal of pain (high catastrophising) and its meaning can be associated with a different generalisation gradient than that associated with a less negative appraisal of pain (low catastrophising). Finally, we explored whether participants would press a button to avoid a highly painful stimulus that they have learned to predict.

Methods:

A priori protocol: The procedure and analysis for this study was published and 'locked' on the Open Science Framework website (https://osf.io/92mkn/). Any deviations from this protocol are stated.

Participants

Based on an *a priori* sample size calculation (effect size, f: 0.3; a power of 80% and α of 0.05), 21 healthy participants were recruited through flyers or word of mouth at the University of South Australia. All potential participants were screened for exclusion criteria before they arrived at the lab and on-site prior to the experiment. Potential participants were excluded if they had any of the following: current pain, persistent pain (defined as pain on most days for longer than 12 weeks), pregnancy, injuries, illness (such as neurological, psychiatric, autoimmune, cold and flu, gastrointestinal), medication (which would alter skin sensitivity, have an analgesic effect or affect vision), heart conditions (including pacemakers, due to the use of electrocutaneous stimuli) or skin conditions (particularly located at the site of stimulation). Additional exclusion criteria were: a lack of proficiency in English, and participation in a classical conditioning experiment in our laboratory within the previous six months.

All participants were informed (both verbally and in writing) that they would receive a range of painful and non-painful electrical stimuli during the experiment and that they could withdraw at any time without judgement. Following screening and an explanation of the experiment, all participants signed a consent form. Participants received an honorarium of AU$20 per hour for their time. The experimental protocol was approved by the University of South Australia, Human Research Ethics Committee (HREC; protocol number: 0000033772).

Stimulus material

The conditioned stimuli (CSs), distractor stimuli (DSs) and control stimulus used in this experiment were taken from the Karolinska Directed Emotional Faces (KDEF) set of face images (www.emotionlab.se/ resources/kdef; Lundqvist, Flykt, & Öhman, 1998). The set contains 4900 images developed for use in perception, attention, emotion, memory and masking experiments. It consists of pictures of 70 individuals (35 female, 35 male; mean age 25 years; age range 20—30 years) with seven different facial expressions per individual (Lundqvist et al., 1998). Images of males with neutral facial expressions were used in this experiment to minimise any modulation of emotions. The KDEF set has been used in other similar conditioning studies (Jensen et al., 2012, 2014) and has been previously validated (see Goeleven, De Raedt, Leyman, & Verschuere, 2008, for a full review).

Conditioned stimuli: The CS+ and CS- images were counterbalanced (i.e. half the participants received one face (face A, Figure 1) as the CS+, and the other face (face E, Figure 1) as the CS-. The other half received the opposite: CS+ (face E) and CS- (face A; Figure 1).

Generalisation stimuli: The generalisation stimuli (GSs) were created by using the Face Film application (version 3.3.20; Yan 2016), run on a MacBook computer, to manipulate face images from the KDEF database. The application allowed elements of the conditioned stimuli to be mixed to create images that vary in similarity between the CS+ and CS- (see Figure 1). Initially, seven blended faces were created. However, an online survey (Talbot, Madden, Jones & Moseley, unpublished) and pilot studies revealed that the distinguishability between the very similar faces was poor, so the number of blended faces was reduced to three.

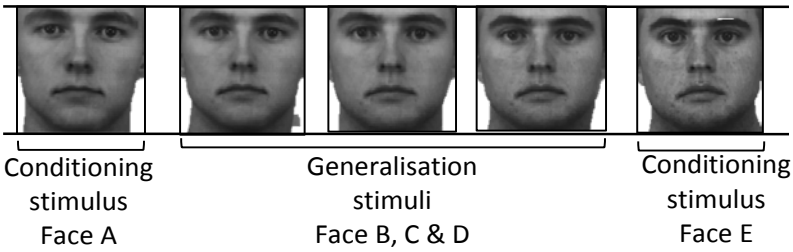


Figure 1. Face image A and Face image E are the two distinct male faces used as the conditioning stimuli. Face images B, C and D are blends of faces A and E, and were used as generalisation stimuli.

Distractor stimuli: Four individual male face images with neutral expressions were used as the distractor stimuli (Figure 2). Four distinct faces were used to increase the difficulty of the task.

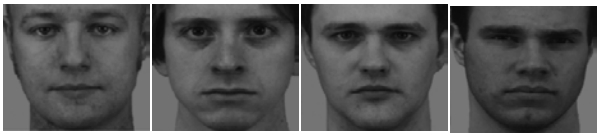


Figure 2. Four distinct neutral male face images used as distractor stimuli.

Control stimulus: One image of a different male face with a neutral expression was used as the control stimulus in the Generalisation phase (pain intensity; Figure 3).



Figure 3. Image of a male face with a neutral expression, used as the control stimulus in the Generalisation phase-pain intensity.

Unconditioned stimuli: Electrocutaneous stimulation was used as the US. Three intensities of stimuli were administered to the ventral surface of the forearm using two types of electrodes: (1) transcutaneous electrodes delivered the low intensity stimulus (referred to as the 'non-painful US'); and (2) intracutaneous electrodes (made in-house) delivered the moderate and high intensity stimuli (referred to as the 'moderately painful US' and 'highly painful US'). The different stimulus intensities assigned to the respective pain ratings were

determined during a calibration process. The different electrodes delivered electrocutaneous stimuli of a similar quality. However, the intracutaneous electrodes enabled higher pain ratings to be elicited at lower intensities. This was important, as both moderately painful US and highly painful US ratings were required.

Materials

Electrodes

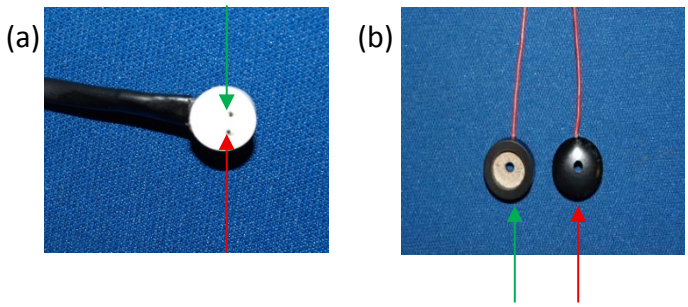


Figure 4. The two types of electrodes used to deliver the stimuli. Image (a) is the intracutaneous electrode and image (b) is the transcutenous electrodes. The green arrow is showing the anode and the red arrow is showing the cathode. The transcutaneous electrodes comprised an anode and a cathode. They were circular plastic devices that could be attached to the surface of the skin with adhesive tape. Conduction gel was used to ensure transmission of the electrical stimulus. The intracutaneous electrodes used in this study were made in-house. The electrode had both an anode and cathode inserted in a small circular plastic cap of 10 mm diameter. The probes projected past the cap by approximately 3 mm. This was designed to ensure the probes indented, but did not pierce the skin, thus making them different from intradermal electrodes.

Electrical equipment: Two constant current stimulators were used to deliver the electrocutaneous stimuli. A DS7A device (SDR Scientific, Power: 12 Va, Freq: 47-63 Hz, Hydeway Welwyn, Garden City, UK) delivered the non-painful US, and a DS7AH device (same details) delivered the moderately painful US and highly painful US.

Programming equipment*:* The experiment was programmed using Affect 4.0 software (Spruyt, Clarysse, Vansteenwegen; Baeyens & Hermans, 2010).

Outcomes

Pain Intensity (primary outcome): During some experimental trials (Table 1) participants rated pain intensity using the Fifty Either Side of Threshold Scale (FESTS; Madden et al., in review). The question was 'Please rate your experience on the appropriate side of the scale', with -50 meaning 'no sensation', +50 meaning 'the most intense pain you can imagine' and 0 meaning 'the exact point at which what you feel transitions to pain'. The written explanation emphasised that ratings between -50 and 0 reflected a non-painful experience and ratings between 0 and 50 reflected a painful one.

Button option (behavioural outcome): During some experimental trials participants had a button press option available to them (Table 1). Participants could elect to push the button or not. The following data regarding the button option was recorded: whether the button was pushed (yes or no) and whether it was appropriate or inappropriate (i.e. correct = CS+; incorrect = CS- or GS or DS- see Figure 6 for further explanation).

US expectancy (manipulation check): During some experimental trials (Table 1), participants rated US expectancy using the 100 mm Visual Analogue Scale (VAS). The question and anchors used to assess US expectancy were, 'How much do you expect the painful stimulus to occur now?', with 0 meaning 'not at all', and 100 meaning 'very much'.

Explanation and practice: The US expectancy and pain intensity scales and the button option (behavioural outcome) had verbal and written explanations. A verbal explanation was provided to participants during calibration. A written explanation of the scales and/or button option was displayed on the screen during experimental trials. For the rating scales, the text explained what participants were rating (e.g. US expectancy or pain intensity) and how to rate it. The written explanation of the button option was used to emphasise three key instructions: (1) Pushing the button would interrupt the highly painful US. (2) Pushing the button would not interrupt the non-painful US or the presentation of face stimuli. (3) At different times during the experiment the button could be pushed either a limited or unlimited number of times, the participant would be informed before the block which option was available. The actual number of pushes available to the participant was not disclosed.

Before the main experiment began, a practice phase commenced during which the scales and button option were displayed on the screen. The participants practiced using a mouse to select US expectancy or pain intensity ratings along a line on the screen, and pushing the keyboard speace bar to activate the button option.

General experimental procedure

Preparation: The ventral forearm was abraded and cleaned, and the transcutaneous electrodes (anode and cathode) were fixed to the skin approximately 40 mm apart using Micropore hypoallergenic medical tape (Figure 5). Conduction gel was then injected, using a blunt needle, into the transcutaneous anode and cathode. The two intracutenous electrodes were placed between the transcutaneous electrodes (Figure 5) and were also attached with Micropore tape. Attention was paid to ensuring that none of the electrodes touched each other. Half of the participants received the highly painful US from location A and the moderately painful US from location B; the other half received the opposite. Participants were seated ≈600 mm away from the screen with their arms in a comfortable position. Participants were asked to minimise any movement to prevent the electrodes from coming loose. A partition prevented the participant from seeing the stimulators or the researcher. The keyboard was placed ≈150 mm from the edge of the table. The mouse was placed ≈150 mm away from the middle of the space bar button, on the right.

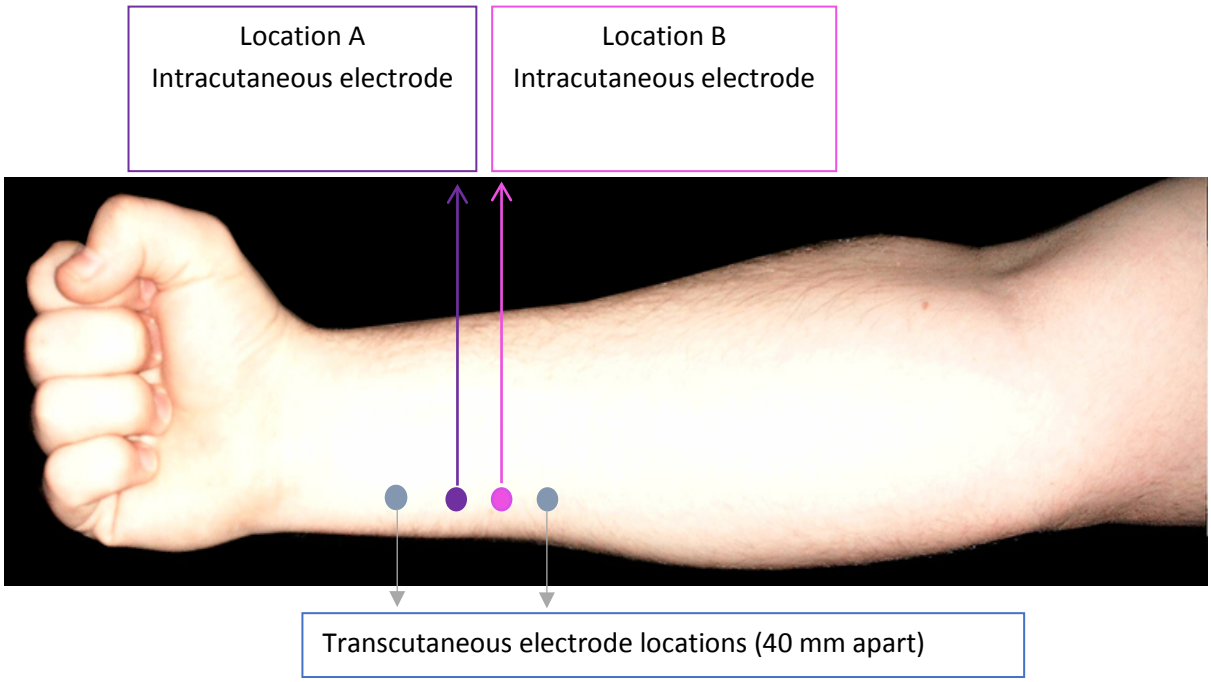


Figure 5. Locations of intracutaneous and transcutaneous electrodes placed on the ventral forearm. Note that, in the case of stimulation via the transcutaneous electrodes, current transmission occurs *between* the two electrodes, such that the percept is located between them – at the site of the two intracutaneous electrodes.

Calibration: An *a priori* protocol was devised for the calibration procedure (see appendix for further details). The calibration procedure involved administering a very low intensity electrocutaneous stimulus to the participant's forearm and then incrementally increasing the stimulus intensity. The participant was asked to rate each stimulus using the FEST scale. A reported intensity rating of 20/50 (moderately painful) was identified for the highly painful US. A reported intensity rating of 5-10/50 (mildly painful) was identified for the moderately painful US. A rating of -40/-50 was identified for the non-painful US. These were then used during the main experimental procedure.

Main experimental procedure

A within-subjects experimental design was used with a classical (trace) conditioning protocol. The study comprised a single 90-minute session consisting of several experimental blocks: Preparation, Calibration, Acquisition blocks 1, 2 & 3, Generalisation block – US expectancy, Re-Acquisition, Generalisation block – pain intensity, Extinction block 1 and Extinction block 2. The procedure is outlined in Table 1.

Table 1. Experimental protocol.

Phase	Trials included												Ratings required		Behavioural outcome
	CS+ $_{\text{High US}}$	CS+ $_{\text{Mod US}}$	CS+$_{\text{alone}}$	CS- $_{\text{Mod US}}$	CS-$_{\text{Low US}}$	CS- $_{\text{alone}}$	GS 1-3 $_{\text{Mod US}}$	GS 1-3 $_{\text{Low US}}$	DS $_{\text{Mod US}}$	DS $_{\text{Low US}}$	DS $_{\text{Alone}}$	Control $_{\text{Mod US}}$	US $_{\text{EXP}}$	Pain $_{\text{intensity}}$	Button option available
	Number of trials														Ratings
ACQ 1	4^T				4^T					8^T					
ACQ 2	4^T				4^T					8^T			16^R		LIMITED
ACQ 3	4^T				4^T					8^T			16^R		LIMITED
GEN. $^{\text{US}}$ expectancy	2^T				2^T			2^TX 3GS		2^T			12^R		LIMITED
Re-ACQ	3^T				3^T									6^R	
GEN $^{\text{Pain}}$ intensity		2^T		2^T			2^TX 3GS		2^T			2^T		1 4^R	
EXT 1			4^T			4^T					8^T		16^R		
EXT 2			4^T			4^T					8^T		16^R		UNLIMITED

Note: Table segments are shaded if, during a block, a particular CS or DS or GS occurred and a particular conditioned response was recorded.

Code	Explanation
ACQ	Acquisition
GEN	Generalisation
EXT	Extinction
US	unconditioned stimulus
T	Trial
R	Rating
CS+ $_{\text{High US}}$	CS+ face image paired with the high-pain US
CS+ $_{\text{Mod US}}$	CS+ face image paired with the moderate-pain US
CS+ $_{\text{Alone}}$	CS+ face image without the US
CS- $_{\text{Mod US}}$	CS- face image paired with the moderate-pain US
CS- $_{\text{Alone}}$	CS- face image without the US
GS 1-3 $_{\text{Mod US}}$	The three different generalisation face images paired with the moderate-pain US
GS1-3 $_{\text{Low US}}$	The three different generalisation face images paired with the non-painful US
DS $_{\text{Mod US}}$	The distractor face images paired with the moderate-pain US
DS $_{\text{Low US}}$	The distractor face images paired with the non-painful US
DS $_{\text{Alone}}$	The distractor face images without the US
Control $_{\text{Mod US}}$	The control face image paired with the moderate-pain US
PAIN $_{\text{intensity}}$	Pain intensity FESTS rating
US_{EXP}	US expectancy VAS rating

Acquisition (US expectancy and button push): The Acquisition phase comprised three blocks of 16 trials each (Figure 6). For the first block, no ratings or button options were available. For the second and third blocks, the outcomes rated were (1) US expectancy and (2) button-pushing behaviour. Stimuli were selected from a pool including CS+, CS- and DS face images (Table 1).

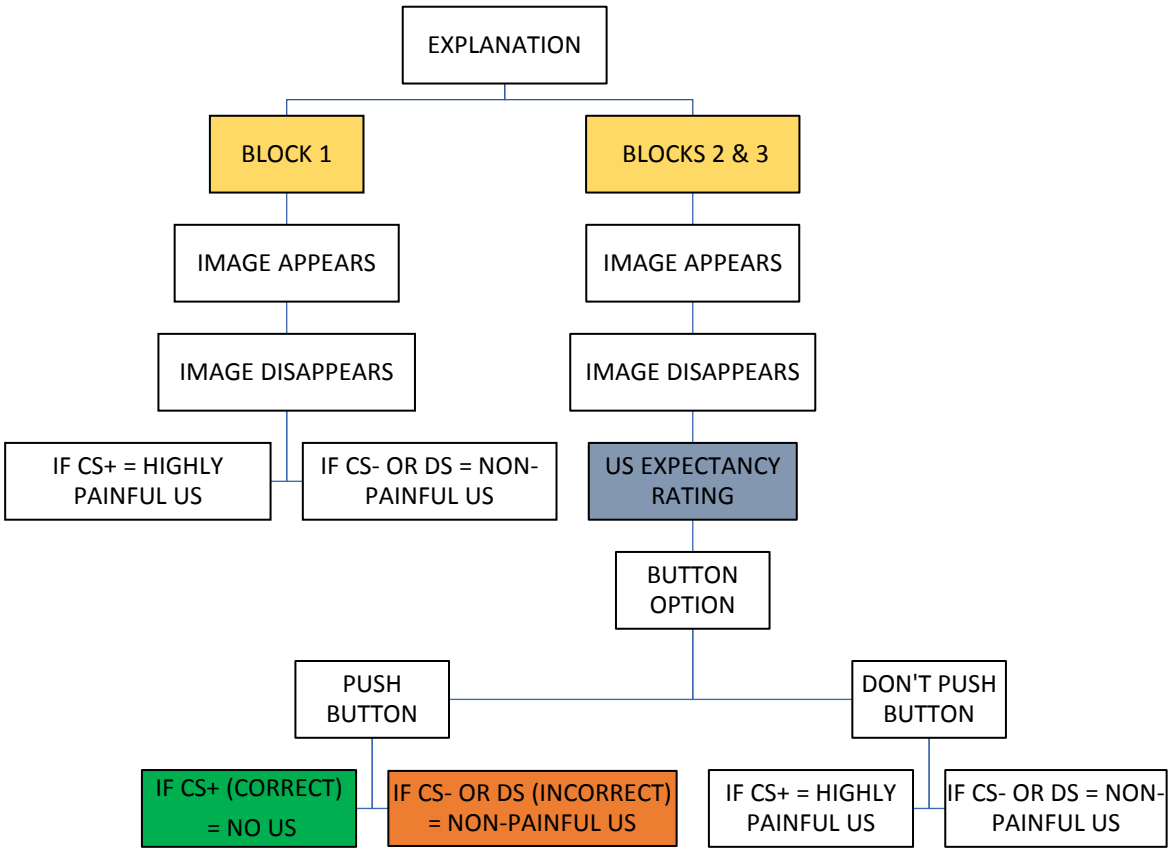


Figure 6. Experimental trial flowchart for Acquisition blocks 1, 2 and 3.

Generalisation block—US expectancy (button option): The Generalisation block – US expectancy comprised one block of 12 trials. The trial flow was similar to the third block of the Acquisition phase (Figure 7). For this block, the outcomes rated were (1) US expectancy and (2) button-pushing behaviour. Stimuli were selected from a pool including CS+, CS-, GS 1-3 and DS face images (Table 1). In this phase, three GS were included and were paired with the non-painful US.

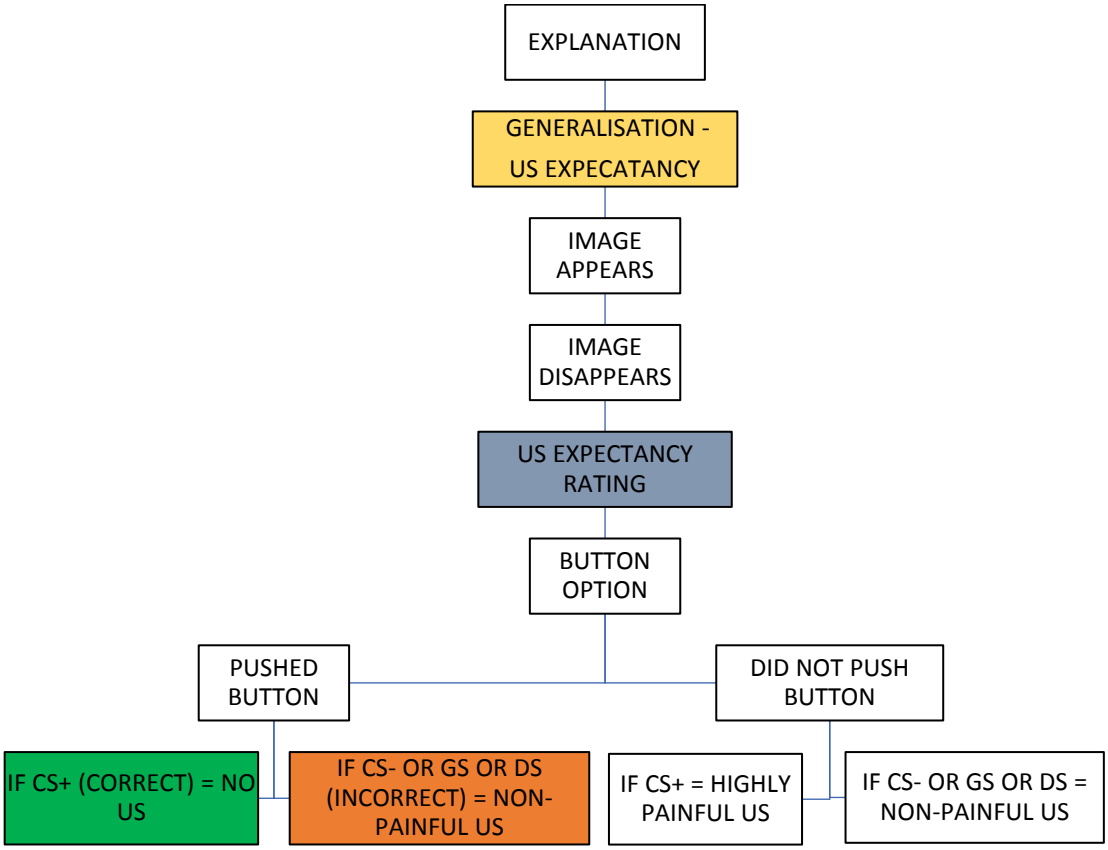


Figure 7. Experimental trial flowchart for Generalisation block – US expectancy.

Re-acquisition (pain intensity): The Re-acquisition phase comprised one block of six trials. Three of the trials involved the CS+ paired with the highly painful US, and the other three trials involved the CS- paired with the non-painful US. Pain intensity was rated using the FESTS, and no button option was available.

Generalisation block—pain intensity (no button option): The Generalisation block – pain intensity block comprised one block of 14 trials. Pain intensity was rated using the FESTS and no button option was available (Figure 8). The stimuli were selected from a pool of CS+, CS-, GS 1-3 and DS face images. All stimuli were paired with the moderately painful US.

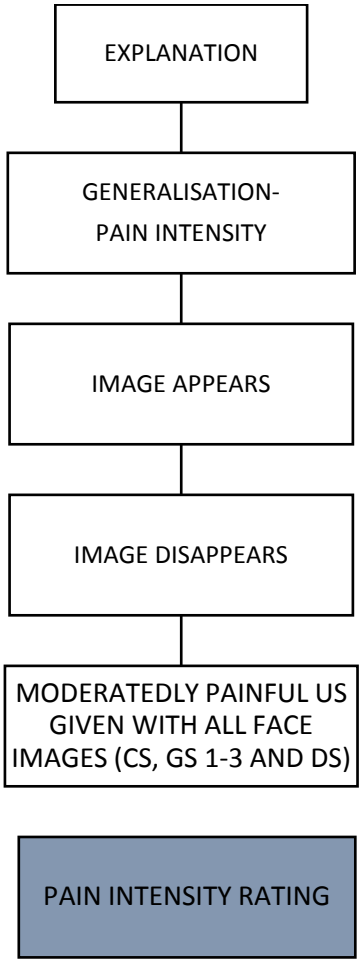


Figure 8. Experimental trial flowchart for Generalisation block – pain intensity.

Extinction block 1 (US expectancy) and Extinction block 2 (US expectancy and button push): Extinction blocks 1 and 2 comprised 16 trials. The trial flow was similar to the third block of the Acquisition phase. The outcome rated for Block 1 was US expectancy and for Block 2 it was US expectancy and button-pushing behaviour. For Block 2, participants were informed that they had unlimited button pushes. Stimuli were selected from a pool including CS+, CS- and DS face images (Table 1). No electrocutaneous stimuli were paired with the visual stimuli (face images) during this phase.

Post-experimental outcomes

After the main experiment, participants performed a manipulation check, were asked questions relating to blinding and the button option, and filled in three questionnaires.

Manipulation check (perceptual acuity): A two-way forced-choice paradigm was used to assess participants' ability to distinguish between the different face images used in the experiment. Participants were shown a series of face pairs displayed one after another. After viewing a face pair, participants rated whether the two faces were the same or different. If correct, then participants were deemed able to distinguish between the pair of faces. If they were incorrect they were deemed unable to distinguish between the faces of that face pair. Participants with more than 80% correct responses were allocated to the high perceptual acuity group. Those 70-79% correct were allocated to the medium perceptual acuity group, and those with less than 70% were placed in the low perceptual acuity group.

Blinding: Subsequent to the manipulation check, participants were asked a question relating to blinding: 'What do you think this study was investigating?' When a vague answer, such as 'pain and faces' was provided, participants were asked to elaborate. Participants were considered naive if they were unable to identify the role the GSs played in the experiment.

Button option (behavioural outcome): Next, participants were asked two questions relating to the button option: (1) 'Did you push the button?', and (2) 'Why did / didn't you?'

Questionnaires: Finally, participants were asked to complete the Fear-Avoidance Beliefs Questionnaire (FABQ), the Neurophysiology Pain Questionnaire (NPQ), and the Catastrophising Assessment Tool (CAT). Each questionnaire has been previously tested and found to be reliable (FABQ: George et al., 2006; Waddell et al., 1993; CAT: Lefebvre, Keefe, Caldwell, Waters, & Ulrich, 2002; NPQ: Catley, O'Connell, & Moseley, 2013).

Statistical Analysis

General approach

All data were entered into a Microsoft Excel spreadsheet (Windows 8, Microsoft Office, University of South Australia, Adelaide). Data were analysed using SPSS Version 21.0 (IBM, 2012).

Normality: Data distributions were analysed using a Shapiro-Wilk test. The following *a priori* parameters were set: 1): If distribution skewness was between 1 and -1, then parametric tests were used. If this parameter was not met, the data were transformed and normality reassessed. After re-analysis, if the data were still not normally distributed, then non-parametric tests were used. 2) Repeated measures ANOVA: if Maulchy's statistic was significant, indicating that sphericity had been violated, then Greenhouse-Geisser-adjusted values were reported.

Manipulation check

Perceptual acuity: To assess whether participants' perceptual acuity of the different face stimuli modulated the US expectancy and pain intensity ratings for the different stimuli, a three-way mixed ANOVA was conducted with a between-subjects factor of group (3 levels: high, medium and low perceptual acuity) and a within-subjects factor of face images (5 levels: CS+, GS1-3 and CS-).

US expectancy: To investigate whether the US expectancy was rated higher for the CS+ than for the other face stimuli, and whether the US expectancy ratings decreased as the similarity of the face stimuli to the CS+ decreased, a one-way repeated measures ANOVA was conducted with a within-subjects factor of face images (5 levels: CS+, CS- and GS1-GS3). Simple contrasts were used to determine the difference between the US expectancy ratings for the CS+ and the CS- and GS 1-3.

Primary analysis

Pain intensity: To investigate conditioning-induced hyperalgesia, a paired *t*-test of the mean pain ratings for the CS+ and CS- stimuli was conducted. To analyse the reliability between the three conditions (CS+ face, CS- face and control face) Cronbach's alpha was used. To investigate whether conditioning-induced hyperalgesia generalised to the GSs, a one-way ANOVA was conducted with a within-subjects factor of face images (5 levels: CS+, CS- and three GSs).

Secondary analyses

Questionnaire data: For FABQ and CAT questionnaires, participants were ranked according to their raw scores. The top eight were categorised as 'high', and the bottom eight as 'low'. To investigate whether differences in fear-avoidance beliefs, and catastrophising were associated with differences in the generalisation of conditioning-induced hyperalgesia, a two-way mixed ANOVA with a between-subjects factor of group (2 levels: high and low) and a within-subjects factor of face images (5 levels: CS+, GS1-3 and CS-) was conducted for both fear-avoidance beliefs, and catastrophising.

Behavioural response—button push: Not all participants used the button option. Moreover, participants failed to use it consistently throughout the experiment. Therefore, the behavioural response data were not analysed. This omission was a deviation from our *a priori* protocol (https://osf.io/92mkn/) but was decided upon before further analyses.

Results

Participant data: Twenty-three healthy participants were recruited. Two were excluded because they failed to rate the maximum electrocutaneous stimulus as highly painful. The remaining 21 participants (11 females, 10 males; age range: 18—55 years; mean ± SD age: 29 ± 9.7 years) completed the experiment.

Rating of unconditioned stimulus: The mean ± SD electrical current used to produce the electrocutaneous stimuli were: 1) highly painful US: 35 ± 18.8 mA; 2) moderately painful US: 21 ± 13.4 mA; and 3) non-painful US: 4.3 ± 2.5 mA.

Blinding: All participants were considered blinded to this study's hypothesis because none identified the role of the GS in the experiment.

Testing of assumptions

US expectancy: A Kolmogorov-Smirnov test statistic of 0.194 (p = 0.00) indicated that US expectancy ratings were not normally distributed, but the distribution skewness statistic was 0.462, which was within the acceptable range defined *a priori*.

Pain intensity: A Kolmogorov-Smirnov test statistic of 0.064 (p = 0.20) indicated that pain intensity ratings were normally distributed.

Fear-avoidance beliefs: A Kolmogorov-Smirnov statistic of 0.135 (p < 0.001) indicated that the data were not normally distributed, but the skewness distribution statistic was 0.237, which was within the acceptable range defined *a priori*.

Catastrophising: A Kolmogorov-Smirnov statistic of 0.164 (p = 0.00) indicated that the data were not normally distributed, but the skewness distribution statistic was 0.294, which was within the acceptable range defined *a priori*. Therefore, parametric tests were used to analyse all data.

Manipulation check: For perceptual acuity and the extent of generalisation of US expectancy, Mauchly's statistic (W = 0.070, p = 0.00) indicated that the assumption of sphericity was violated; therefore, Greenhouse-Geisser-corrected statistics were reported.

There was no significant difference between the different perceptual groups in the US expectancy ratings for the different face stimuli ($F_{(11, 34)}$ = 1.820, p = 0.277; Figure 9)

Perceptual acuity and the extent of generalisation of pain intensity ratings: Mauchly's statistic (W = 0.294, p = 0.018) indicated that the assumption of sphericity was violated; therefore, Greenhouse-Geisser-corrected statistics were reported. There was no significant difference in the pain intensity ratings for the different face stimuli between the different perceptual groups ($F_{(5, 48)}$ = 1.424, p = 0.230; Figure 10).

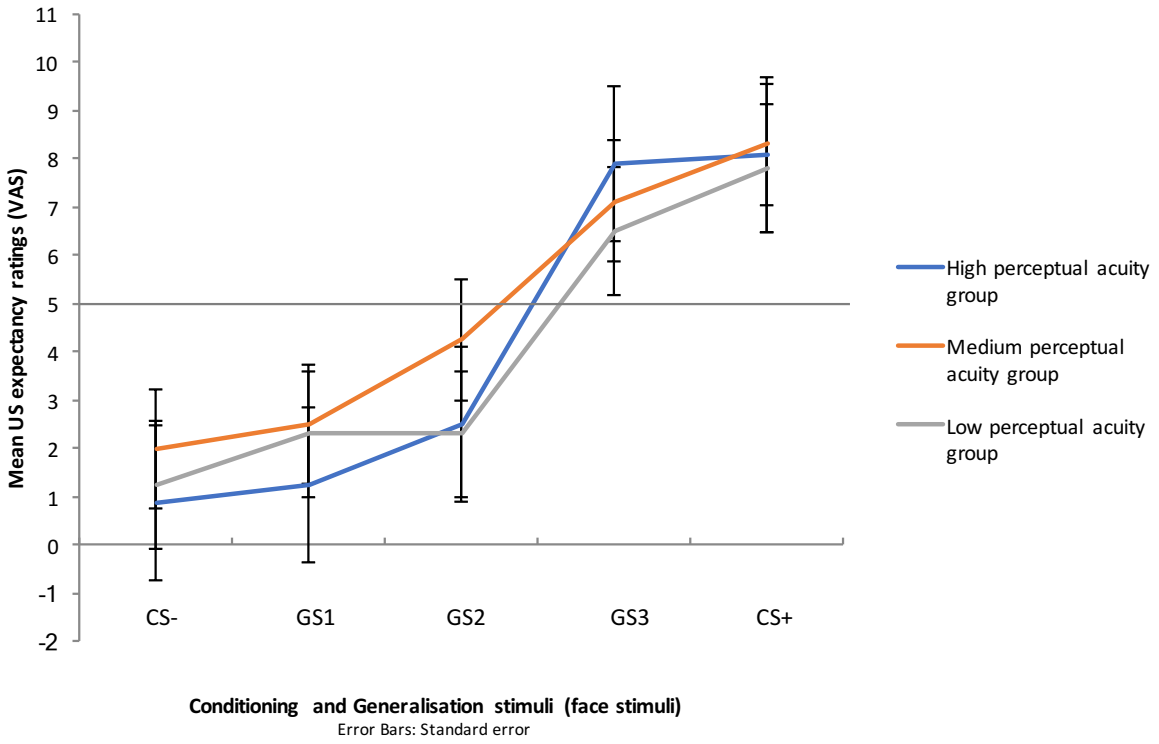


Figure 9. Differences in the extent of generalisation of conditioned US expectancy ratings for the high, medium and low perceptual acuity groups.

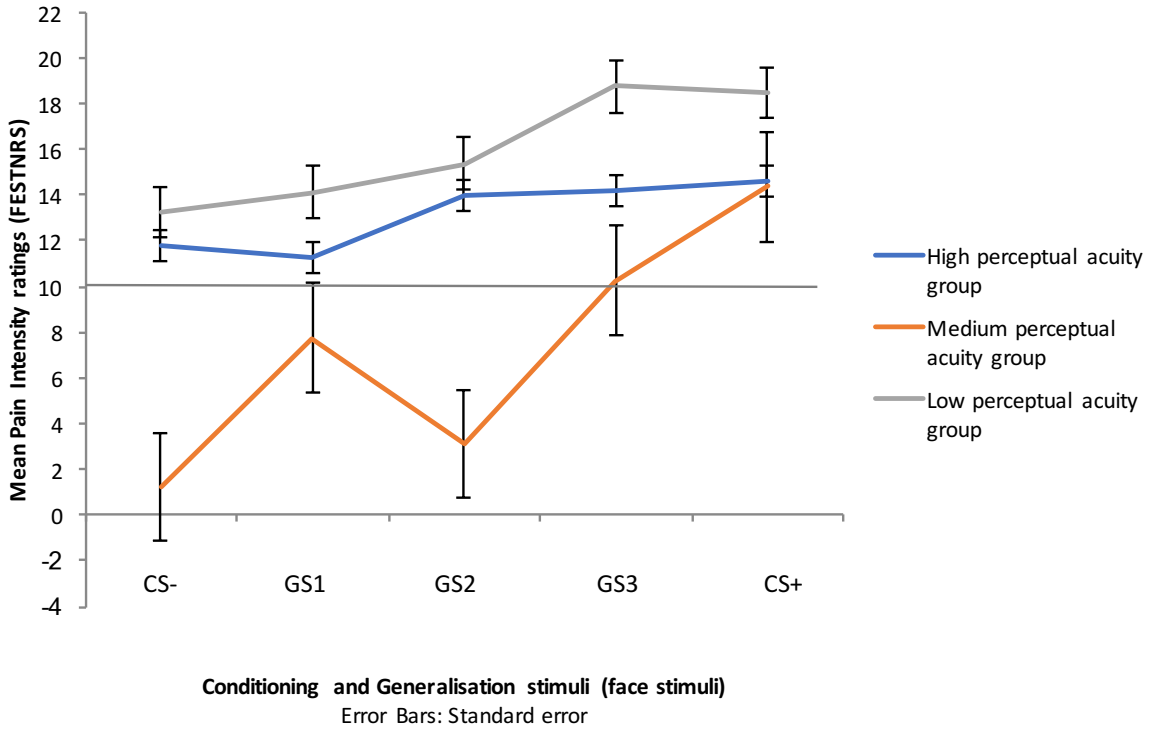


Figure 10. Differences in the extent of generalisation of conditioning-induced hyperalgesia for the high, medium and low perceptual acuity groups.

US expectancy: Mauchly's statistic (W = 0.073, p = 0.00) indicated that sphericity was violated; therefore, Greenhouse-Geisser statistics were reported. The US expectancy ratings differed for the face stimuli ($F_{(4, 80)}$ = 57.801, p = 0.00; Figure 11). Simple contrasts showed a significant difference between the US expectancy ratings for the CS+ and for the other face stimuli: CS- ($F_{(1,20)}$ = 106.6, p = 0.00), GS1 ($F_{(1,20)}$ = 83.5, p = 0.00), GS2 ($F_{(1,20)}$ = 22.2 p = 0.00) and GS3 ($F_{(1,20)}$ = 7.32, p = 0.014).

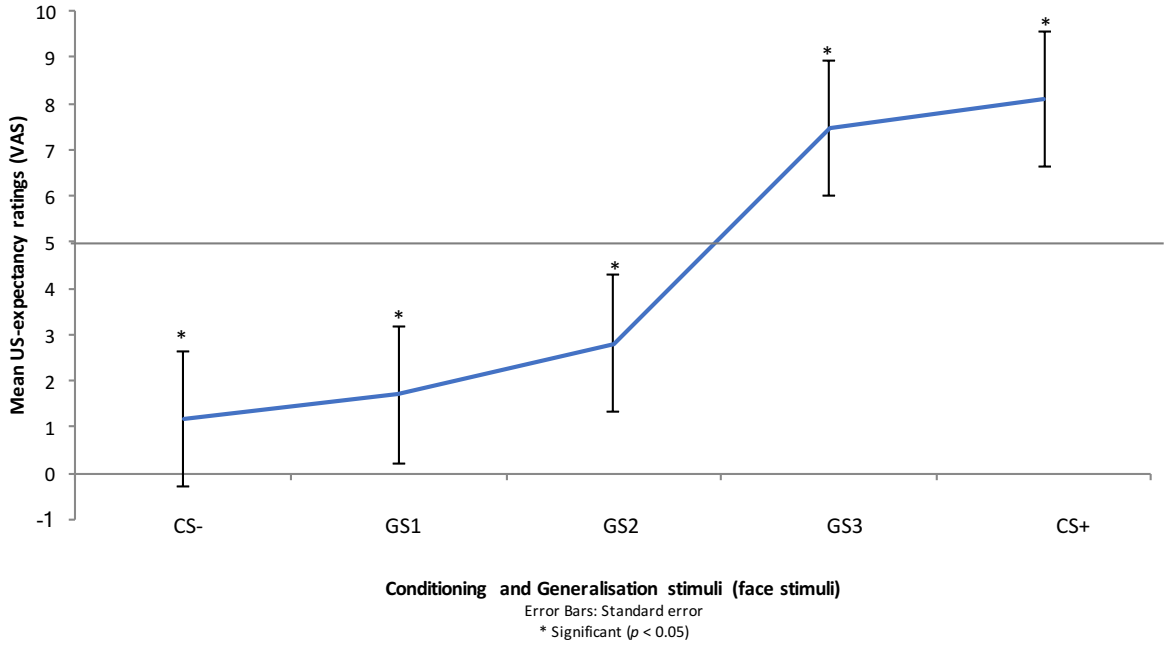


Figure 11. The extent of generalisation of conditioned US expectancy. Participants' mean ± SE US expectancy ratings for each face stimulus.

Primary analysis

Generalisation of conditioning-induced hyperalgesia: There was a significant difference between the mean pain intensity rating of the CS+ (mean ± SD = 15.3 ± 9.7) and that of the CS- (mean ± SD = 10.1 ± 9.9; $t_{(20)} = 2.68$, $p = 0.015$). That is, participants rated the moderately painful stimulus more painful when paired with the CS+ than when paired with the CS- or GS1. The reliability within the three conditions (CS+ face, CS- face and control face) was shown to be high (Cronbach's alpha = 0.841). Mauchly's statistic ($W = 0.287$, $p = 0.006$) indicated that sphericity has been violated; therefore, Greenhouse-Geisser values were reported. The pain intensity ratings differed for the different face stimuli ($F_{(3, 53)} = 3.90$, $p = 0.017$). Simple contrasts showed a significant difference between the pain intensity ratings for the CS+ and the CS- ($F_{(1, 20)} = 7.16$, $p = 0.015$) or the CS+ and GS1 ($F_{(1, 20)} = 9.71$, $p = 0.005$). No significant difference was found between the pain intensity ratings of the CS+ and GS2 ($F_{(1, 20)} = 2.34$, $p = 0.14$) or CS+ and GS3 ($F_{(1, 20)} = 1.03$, $p = 0.32$; Figure 12).

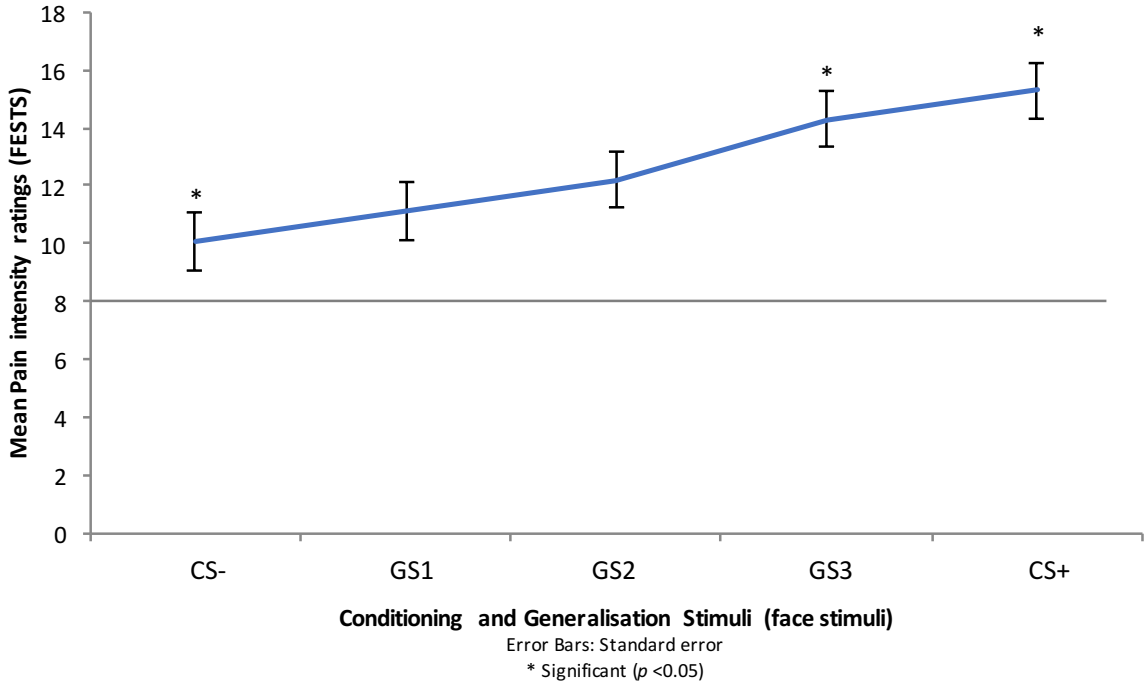


Figure 12. The extent of generalisation of conditioning-induced hyperalgesia. Mean pain intensity ratings for each face image stimulus.

Secondary analysis

Fear-avoidance beliefs: Mauchly's statistic of 0.161 (p = 0.007) indicated that sphericity had been violated; therefore, Greenhouse-Geisser values are reported. The mean pain intensity ratings of the different face stimuli did not differ between the two groups (high FAB group vs. low FAB group; $F_{(4, 56)}$ = 0.408, p = 0.716; Figure 13).

Catastrophising: Mauchly's statistic of 0.250 (p = 0.047) indicated that sphericity had been violated; therefore, Greenhouse-Geisser values are reported. The mean pain intensity ratings for the different face stimuli did not differ between the two groups (high CAT group vs. low CAT group; $F_{(4, 56)}$ = 1.245, p = 0.303; Figure 14).

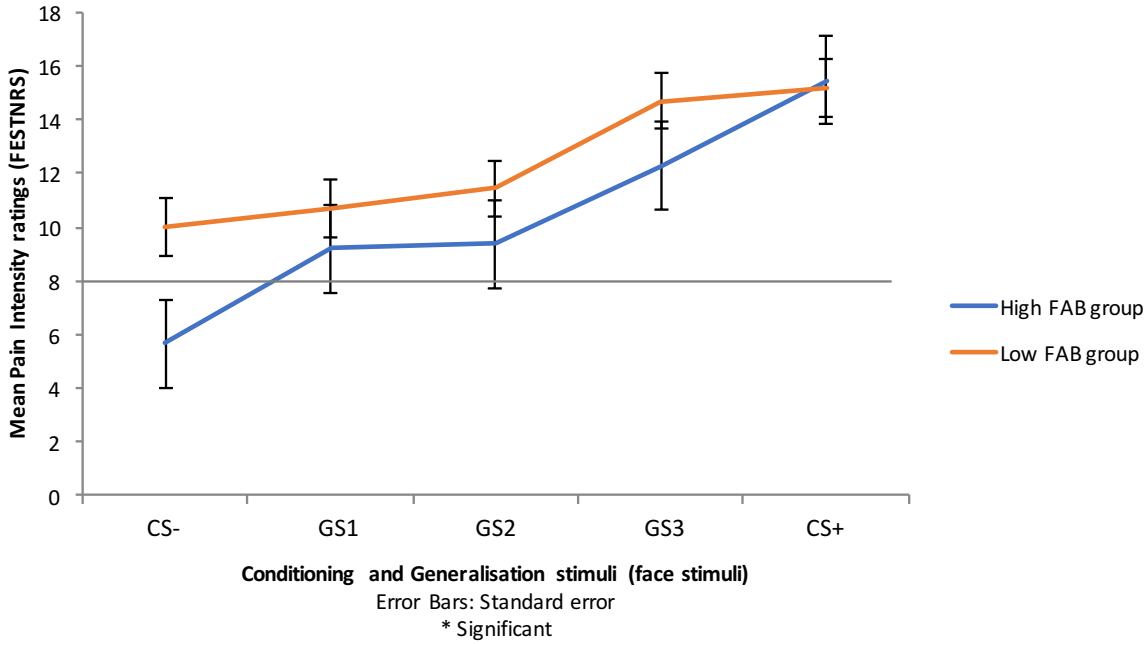


Figure 13. The extent of generalisation of conditioning-induced hyperalgesia between the high and low fear-avoidance beliefs (FAB) groups.

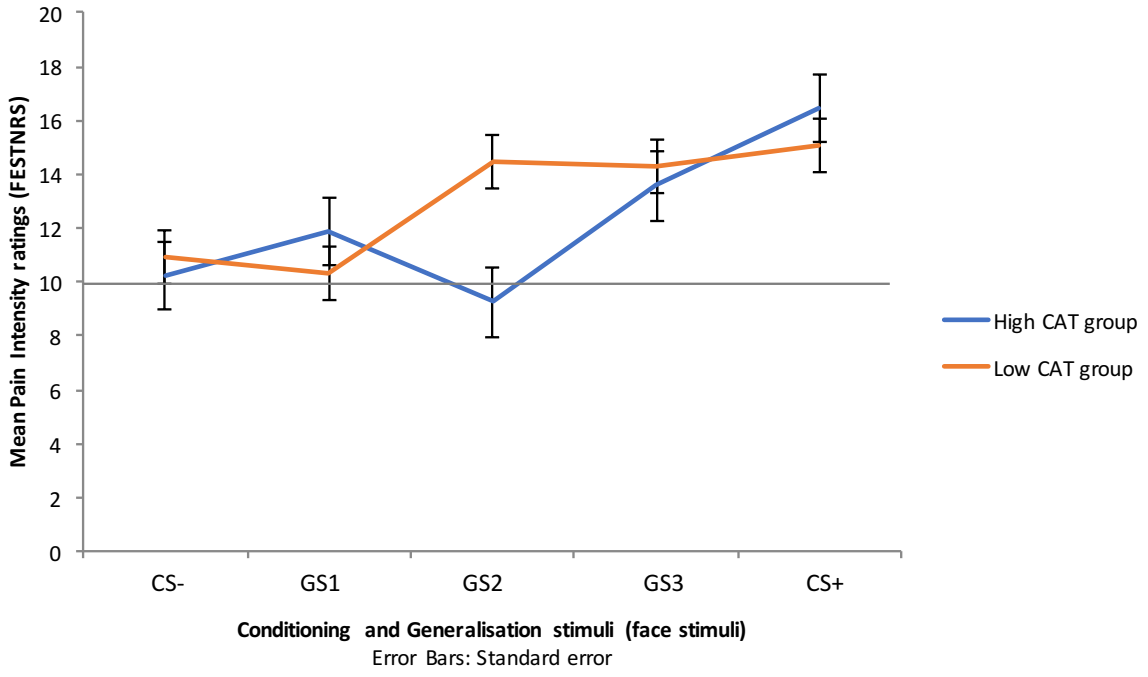


Figure 14. The extent of generalisation of conditioning-induced hyperalgesia between the high and low catastrophising (CAT) groups.

Participant responses regarding the button option:

A total of 14 participants used the button. However, not all participants who pushed the button in the Acquisition phase pushed it in the Extinction phase, and vice versa. Moreover, some used the button only once throughout the experiment, while others used it multiple times. The seven participants who did not use the button said that they didn't feel the need to use it as they knew the highly painful stimulus was temporary. The 14 participants who did use the button did so to avoid the highly painful stimulus. From the 14 participants who used the button option, two interesting responses were revealed. First, two participants stated that when the button option was limited, they sometimes chose not to use it despite the fact that they did expect a highly painful US. Second, two participants stated that when they had unlimited button pushes, they still pushed it even if they weren't expecting the highly painful US, just to be on the "safe side".

Discussion

We hypothesised that a painful stimulus would be rated as more painful if it was preceded by a CS+ than if it was preceded by a CS- (i.e. classical conditioned-induced hyperalgesia). Moreover, we hypothesised that the magnitude of conditioning-induced hyperalgesia would decrease as the similarity of the novel faces and the CS+ face decreased. The results support both hypotheses.

The results of this study confirm the results of other studies investigating conditioning-induced hyperalgesia, and extend them with regards to the generalisation of conditioning-induced hyperalgesia (Harvie et al., 2016; Jensen et al., 2012, 2014). Certainly, stimulus generalisation is not new—there is a large base of associative learning studies that have shown that a classically conditioned response will generalise to similar but novel stimuli (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015; Grant & Schiller, 1953; Lissek et al., 2008; Meulders et al., 2014; Meulders, Harvie, Moseley, & Vlaeyen, 2015; Pearce, 1987; Schechtman, Laufer, & Paz, 2010). This phenomenon is thought to be advantageous to survival, because it allows an individual to predict potentially dangerous stimuli and avoid them (Vlaeyen, 2015). However, a novel take on stimulus generalisation is outlined by the imprecision hypothesis of chronic pain (Moseley & Vlaeyen, 2015). This hypothesis proposes

that the more imprecise the encoding and cortical representation of a painful event is, the greater the potential for more stimuli to resemble the painful event and therefore evoke a pain response (Moseley & Vlaeyen, 2015). While the current study did not show conditioned pain or allodynia, it did show that a non-nociceptive stimulus (face image) that was associated with a highly painful stimulus could subsequently amplify a pain response. Moreover, it shows that this association influenced how participants then responded to similar but novel faces, such that the less similar the face was to the CS+, the less pain they reported. That is, participants reported the moderately painful US paired with the stimuli that were similar to the CS+ as more painful than stimuli that were less similar, despite the fact that they had never before experienced the similar stimuli as painful.

While the clinical use of face images in the context of pain is not as ecologically-valid as using interceptive stimuli such as movements, the stimuli are still more valid than arbitrary stimuli such as shapes (Domjan, 2005). Indeed, when investigating a real-life phenomenon such as pain, a functional perspective on the classical conditioning paradigm has shown a stronger conditioned effect than when using arbitrary stimuli which are not reflective of real-life (Domjan, 2005). For example, a fear conditioning study showed a stronger fear response when using biologically-relevant CSs of snakes or spiders than of mushrooms (Öhman & Mineka, 2001, 2003). Therefore, future studies investigating the role of associative learning in pain should consider the biological relevance of the CS, GS and US. Moreover, the more biologically-relevant the CS, GS and US are, the more clinically applicable the findings will be.

Our secondary analyses showed that the extent of generalisation of conditioning-induced hyperalgesia did not differ between the high and low FAB and CAT groups. This was unexpected, because several studies have shown how factors such as previous experience, beliefs, and expectation can alter pain perception (George et al., 2006; Moseley & Vlaeyen, 2015; Volders, Boddez, De Peuter, Meulders, & Vlaeyen, 2015; Waddell et al., 1993). Moreover, high catastrophising has been linked with high reported pain intensity (Picavet, Vlaeyen, & Schouten, 2002; Sullivan, Stanish, Waite, Sullivan, & Tripp, 1998). However, there are two possible explanations for these unexpected results. First, the allocation of participants to the high FAB group and high CAT group was based on a comparison between the participants in this study. However, the ‘high’ group’s scores may not be considered

'high' for a population with persistent pain (Waddell et al., 1993). Second, the context is different. As hinted by participants in regards to the button option, knowledge of the safety, brevity and control of the painful experimental stimulus reduces its threat value (Coghill, McHaffie, & Yen, 2003). Therefore, this knowledge might reduce the effects of the fear-avoidance beliefs and catastrophising shown in the questionnaires.

Certain limitations of this study should be considered when interpreting the results. A limitation of this current study was that not all participants used the button option, and those who did, did so inconsistently. One potential explanation for this is that the need for the button option appeared to be somewhat negated by the knowledge that the painful stimulus was not threatening (Tabor, Catley, Gandevia, Thacker, & Moseley, 2013). Differences in perceptual acuity did not appear to significantly alter the extent of generalisation of conditioning-induced hyperalgesia. However, the limitation that participant's perceptual acuity was only measured after the experiment affected the reliability of the results (Vervliet & Van, 2015).

Some strengths of this study are that: (1) an *a priori* sample size was calculated. (2) A protocol was devised and lodged before the experiment commenced. (3) Both the verbal and written explanations were devised *a priori* and standardised throughout the experiment. Furthermore, the standardised written instructions were used to minimise coaching of participants during the experiment. (4) Participants were blinded to which electrode delivered which US (the highly painful US or the moderately painful US). (5) The researcher was blinded to the participant's responses because all responses were recorded by a computer. (6) Whether particpants could distinguish between the CS and GS was assessed before the experiment began, via an e-survey (Talbot, Madden, Jones & Moseley, unpublished) and pilot testing. Additionally, whether participants could distinguish between the CS and GS was assessed after the experiment was statistically analysed. While this is more frequently assessed in fear and avoidance classical conditioning studies, the construct of perceptual discrimination has received less attention and, therefore, has been assessed less often in classical conditioning studies in the context of pain (Struyf, Zaman, Vervliet, & Van Diest, 2015; Vervliet & Van, 2015).

Conclusion

The current study showed that conditioning-induced hyperalgesia can generalise to similar yet novel stimuli in healthy participants. These findings add to the existing body of evidence for classical conditioning-induced hyperalgesia by demonstrating that *generalisation* of such classically conditioned-hyperalgesia. This current study also highlights the possible role of associative learning in hyperalgesia.

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Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

8 Thesis discussion

References

Publications, presentations and awards

Statements of authorship

Appendices

8. Thesis discussion

This thesis investigated the assumptions outlined in the imprecision hypothesis of chronic pain ('imprecision hypothesis') regarding the generalisation of conditioning-induced hyperalgesia and perceptual discrimination. This chapter will review how the studies contained in this thesis investigated these assumptions.

Section 8.1 discusses how the findings of this thesis relate to the imprecision hypothesis.

Section 8.2 examines the importance of the stimulus, response and reinforcer in classical conditioning.

Section 8.3 discusses the limitations of this work.

Section 8.4 discusses the clinical implications.

Section 8.5 presents the contributions of this thesis to the field.

8.1 The imprecision hypothesis of chronic pain

A painful event involves multiple sensory inputs and contextual cues. The imprecision hypothesis of chronic pain proposes that nociception serves as an unconditioned stimulus, while the other sensory inputs and contextual cues serve as an conditioned stimulus. Repeated association between nociception and the sensory inputs and contextual cues provides an opportunity for associative learning. The imprecision hypothesis proposes that the precision with which the compound stimulus is encoded and represented in the brain determines the degree to which the response (pain) will generalise to other similar compound stimuli (Moseley & Vlaeyen, 2015).

8.1.1 Pain as a classically conditioned response

Conceptualising pain as a *response* is consistent with contemporary thought in the pain sciences, but it is not universally supported. This situation was clearly evident in peer response to the publication of the imprecision hypothesis (Fuchs, Becker, Kleinbohl, Diers, & Flor, 2015). Criticisms included: 1) the hypothesis is not novel, because many classical conditioning studies have shown how a CS can contribute to pain, and 2) pain is a stimulus that cannot be conditioned. The response by Moseley and Vlaeyen (2015) highlighted that the imprecision hypothesis was indeed based on the classical conditioning literature; however, the fundamental difference was the role that pain was assigned. That is, in the imprecicion hypothesis framework, pain is viewed as an output triggered by a compound stimulus, rather than '…a readout of nociceptive input' (Moseley & Vlaeyen, 2015). Moreover, the imprecision hypothesis proposes that because pain is a compound stimulus, the CS that could be imprecisely encoded is constituted of the sensory inputs or contextual cues that accompany nociception. One might refer to Wall and McMahon's (1986, p. 255) observation that, "the labelling of nociceptors as pain fibres was not an admirable simplification but a most unfortunate trivialisation". Clinically, reconceptualising pain in this way seems to be occurring. In 2015, 86.4% of respondents to an online survey of practising healthcare clinicians agreed with the statement "Pain can occur without nociception" (Madden & Moseley, 2016, p. 168), whereas, in a previous survey done in 2004, only 44% had agreed with that idea (Madden & Moseley, 2016). Despite the theoretical strength of the concept that pain is possible with or without nociception because it reflects a protective motivational feeling (Tabor et al., 2017), the current thesis demonstrated *mixed* results

regarding conditioning-induced hyperalgesia (with Chapter 5 demonstrating null findings and Chapter 7 demonstrating generalisation of conditioning-induced hyperalgesia).

8.1.2. *Evidence that does not support the imprecision hypothesis of chronic pain*

This thesis raised problems associated with the imprecision hypothesis. For example, Experiment 1 (contained in Chapter 5) did not show conditioning-induced hyperalgesia (and therefore, obviously, could not show *generalisation* of conditioning-induced hyperalgesia). One critical issue that may explain this situation concerns the ecological validity of experimental pain studies. In a laboratory situation, researchers can never fully replicate the threat value of a nociceptive stimulus, because of the inherent safety requirements of an experimental situation (Moseley & Butler, 2017; Moseley & Vlaeyen, 2015; Price et al., 1987). That is, participants volunteer for these studies, are usually compensated, are reassured that the procedure is safe, are sometimes informed of the number of times they may receive the painful stimulus, are informed that they can withdraw from the experiment at any time, and receive countless other more or less obvious safety cues. The researchers also know the nature, intensity and true danger posed by the noxious stimulus. There is a vast body of literature demonstrating the modulatory effect of such factors on pain, but also on protective outputs that, like pain, are not under voluntary control, and that occur outside of awareness (see Moseley & Butler, 2017, for an extensive review). For example, a study by Sambo, Forster, Williams, and Iannetti (2012) illustrated how change in threat can modulate an automatic protective response. The study examined the hand-blink reflex (HBR). The blink reflex (BR) is elicited by delivering an electrical stimulus to the trigeminal area of the face, while the HBR is elicited by delivering an electrical stimulus to the median nerve at the wrist (Sambo, Liang, Cruccu, & Iannetti, 2012). This reflex is described as a '…primitive and involuntary motor response' to danger (Sambo et al., 2012, p. 12921). This study demonstrated that when the hand is stimulated closer to the face, the recorded HBR is greater than when the hand is away from the face. Moreover, when a thin wooden screen was placed between the hand and face, the HBR was smaller than when there was no wooden screen (Sambo et al., 2012). This study illustrates that safety cues can influence the magnitude of the response. Therefore, failure to replicate threat, which, according to contemporary pain theory (Moseley & Butler, 2015; Tabor, Keogh, & Eccleston, 2016) is the fundamental component that the brain weighs up when determining whether pain will be

helpful, directly affects a researcher's ability to closely mimic real-world pain (Moseley & Butler, 2015, 2017).

This problem of threat has two potential solutions. First, researchers could conduct a observational cross-sectional clinical study, whereby people who present with acute pain are followed, variables are measured and controlled for and outcome measures are taken. For example, people with an acute foot injury could be followed along their trajectory. Variables could be accounted for by using pedometers to measure steps taken and other outcome measures that examine personality through to affective state could be taken. Researchers could then examine whether a movement such as lifting the toes up towards the leg (i.e. dorsiflexion) could become associated with nociception and eventuate in dorsiflexion eliciting pain without nociception. Demonstrating the absence of nociception would be difficult but it might be possible to use microneurographic techniques (Macefield, Gandevia, & Burke, 1990; Vallbo, Hagbarth, & Wallin, 2004), or possibly even spinal magnetic resonance imaging (MRI).

The second solution may be to manipulate threat. That threat can be modulated has been demonstrated in previous studies (Arntz & Claassens, 2004; Moseley & Arntz, 2007; Wiech et al., 2010). These studies suggest two possibilities: one, that the brain weighs up the threat value of the different sensory and contextual cues and responds accordingly and, two, that it is plausible to modulate threat. It is possible that pairing both a classical conditioning paradigm and a threat modulation would help to overcome this problem. This might be achieved by pairing a second CS to an already paired CS and US. This is unconventional. Previous studies have shown that when this was undertaken, the second new stimulus that is added usually does not elicit the conditioned response. This is often referred to as blocking (Domjan, Cusato, & Krause, 2004). However, some evidence (Byrne, Baxter, Buonomano, & Raymond, 1990; Kamin, 1969; Köksal, Domjan, & Weisman, 1994; Mackintosh, 1975; Pearce & Hall, 1980) demonstrates that if the second new CS is biologically relevant to the previously paired CS and US, then it is resistant to a blocking effect. Therefore, it is plausible that a second CS that modulates threat could be added to a previous pairing of movement or a tactile conditioned stimulus and nociceptive unconditioned stimulus. However, the previous pairing of the CS and US must be established before an additional second CS can be added to the framework.

8.1.3 Evidence that supports the imprecision hypothesis of chronic pain

Generalisation is key to the imprecision hypothesis, which proposes that, if a painful event is imprecisely encoded, it will also be imprecisely represented in the brain, which can then result in generalisation (Moseley & Vlaeyen, 2015). This thesis supported some of the assumptions of the imprecision hypothesis. First, it supported the assumption that hyperalgesia can be a classically conditioned response to the *rest of the sensory inputs* that accompany the compound a nociceptive stimulus, and second, that conditioning-induced hyperalgesia generalised to similar but novel stimuli. The evidence in Chapter 7 showed, experimentally, that conditioning-induced hyperalgesia can be elicited by different face stimuli. This chapter highlighted that a visual input (specifically visual images of faces) can be associated with nociception, such that an already painful stimulus was reported as more painful when paired with the face image previously associated with the painful stimulus. It was also found that conditioning-induced hyperalgesia generalised to stimuli that were similar, but which had not been previously experienced as painful. This demonstrated that this association was not mutually exclusive but rather had the potential to generalise to other similar stimuli. This may be observed clinically in people with acute pain, where pain is elicited by several similar movements rather than exclusively by a single one (Zaman et al., 2015, 2016). This is also observed in people with persistent pain. However, in this situation, the pain is elicited by a much broader array of movements, which appear to be less and less similar to the initial movement (Croft, Rigby, Boswell, Schollum, & Silman, 1993; Moseley & Vlaeyen, 2015; Price, 1999).

Chapter 5 provided preliminary evidence that awareness of the association between the CS+ and the painful US affects generalisation. By being unaware of this association, participants had as much as a 30% increased chance of expecting that even the CS- would be painful. Essentially, the less aware the participants were that the CS+ was paired with the painful US, the flatter their US expectancy generalisation gradient was. It is interesting to note that the results from Chapter 5, regarding contingency awareness and generalisation in healthy participants, were similar to those previously observed in people with fibromyalgia (Meulders et al., 2017) and people with chronic unilateral hand pain (Meulders et al., 2014). A study of people with fibromyalgia demonstrated that, in comparison to healthy controls, those with fibromyalgia failed to identify potentially dangerous stimuli (i.e. that the CS+ was predictive of the US; Meulders et al., 2017). Interestingly, FM patients also failed to identify

safety cues (i.e. that the CS- was predictive of no US) in comparison to healthy controls. The generalisation gradients were much flatter for the FM patients than they were for the healthy controls. A similar trend regarding failure to identify safety cues was observed in patients with chronic unilateral hand pain (Meulders et al., 2014).

One possible explanation for why a difference in awareness influences the generalisation of US expectancy is derived from the imprecision hypothesis of chronic pain (Moseley & Vlaeyen, 2015). Within a healthy population, it is possible that the "unaware" group reflect the process where the initial painful event (CS+ paired with the painful-US) is not encoded precisely (Moseley & Vlaeyen, 2015). Failure to learn the distinct association may blur the representation of the CS in the brain, making the representation of the CS more malleable. This malleability may increase the potential for less similar GSs to be incorporated into the CS representation. The less precisely the CS is represented in the brain, the greater the likelihood that GSs that share only a few characteristics with the true CS can be perceived as the CS and evoke a similar CR.

Chapter 3 sought to determine whether cognitive manipulations could selectively and differentially modulate the sensory and affective dimensions of pain. Interestingly, despite wide acceptance that the sensory and affective dimensions of pain can be selectively and differentially modulated (Hofbauer et al., 2001; Price, 1999, 2000; Price & Dubner, 1977), the evidence that the two pain dimensions can be differentially modulated using cognitive manipulations is limited. This finding highlighted that in order to investigate the role of affect in the imprecision hypothesis, more work is required to determine the best method for selectively and differentially modulating the two dimensions of pain. Differentially modulating these two dimensions would allow for investigation of one particular prediction of the imprecision hypothesis—that painful events in which affective characteristics dominate sensory ones—would result in greater generalisation of conditioning-induced hyperalgesia or allodynia. This prediction is based on the notion that the precision of sensory encoding of an event is reduced under high levels of affect. The initial intention of this thesis was to determine whether this effect could be demonstrated using classical conditioning paradigms. A systematic review was undertaken to inform the best method of differentially modulating affect. This review showed that differential modulation was much less straightforward than the prevailing view suggested. The findings of the systematic review

resulted in a shift in direction for the thesis, to include an investigation of the assumptions of generalisation.

Preliminary exploratory evidence from Chapter 5 showed that low positive affect and high negative affect states were associated with differences in the extent of generalisation of conditioning-induced hyperalgesia. That is, the group with low positive affect and the group with high negative affect showed more generalisation. These differences were consistent with the imprecision hypothesis assumption. These preliminary findings raised two possibilities. First, as outlined in the imprecision hypothesis, a heightened negative affect state during a painful event may lead to less precise encoding of that event and therefore increased generalisation. Second, secondary affect may contribute to the overall pain experience, such that when it is reported as a unitary experience, the heightened negative affect leads to an overall greater experience of pain (Price, 1999). That is, in Chapter 5 it is possible that the intensity or sensory dimension of pain experienced by the participants who are in the low negative affect group may be similar to those in the high positive affect group. However, the unpleasantness or affective dimension of pain experienced may be greater in the low negative affect group, leading to a higher overall report of pain for both the conditioned and generalisation stimuli. Price (1999) outlined that often, pain intensity remains the same, but the longer it persists, the more unpleasant it becomes. This it thought to be involved with the contribution of secondary affect, where the meaning and long-term implications of pain can heighten its unpleasantness (Price, 1999). Anecdotal clinical observations often report that people with persistent pain describe the affective dimension of pain more readily than its sensory dimension (Price, Barrell, & Gracely, 1980; Price et al., 1987). It is possible that what is being observed experimentally is this influence of secondary affect.

8.2 The importance of the stimulus, response and reinforcer

Comparing Chapters 5 and 7 of the current thesis with the wider literature (Domjan, 2005, 2014; Esber & Haselgrove, 2011; Haselgrove, Esber, Pearce, & Jones, 2010), particularly with studies that have supported conditioning-induced hyperalgesia (Harvie et al., 2016; Jensen et al., 2012, 2014; Madden, Harvie, et al., 2016) or conditioning-induced allodynia (Madden, Bellan, et al., 2016), suggests that aspects of the stimulus, response and reinforcer might be critical determinants of associative learning studies, particularly those that use classical conditioning (Brush, 2014; Rescorla, 2014).

8.2.1 The conditioned stimulus and the unconditioned stimulus.

The temporal relation between the CS and US is a prominent and influential feature in classical conditioning (Lavond & Steinmetz, 2003). Three different temporal arrangements exist within classical conditioning. These are: simultaneous (the CS and US coincide), delayed (the CS onset is slightly before the US onset) and trace (a gap between the CS offset and the US onset; Lavond & Steinmetz, 2003). *Trace conditioning* requires the participant to recall the memory trace of the CS and associate it with the US that follows. *Trace conditioning* employs a different memory system from that employed by delayed or simultaneous conditioning (Lavond & Steinmetz, 2003). However, when considering a painful event, input from nociceptive-specific neurones will arrive later at cortical targets, because there are several steps at which the message is processed (Woolf, 2009). The difference in timing is likely to be small but may still be sufficient to develop a predictive capacity, at least at a synaptic level (Lawson, 2002). However, this timing differential between nociception and the other sensory input is not detectable at a systems level—one of awareness—which is one reason to consider that, at this level, the 'dangerous event package' reflects a compound stimulus (Moseley & Vlaeyen, 2015).

There was a significant difference between the trace intervals used in the two conditioning studies contained in this thesis and those used in other studies investigating conditioned hyperalgesia. Most previous studies used a zero second trace interval (as opposed to the three seconds used in the two current studies) and demonstrated conditioning-induced hyperalgesia (Jenewein et al., 2013; Jensen et al., 2012, 2014, 2015; Ploghaus et al., 2001). That the studies reported in this thesis used longer trace intervals difference is potentially

problematic, because the majority of the studies that have investigated the effect of different trace intervals have highlighted that learning declines as the trace interval lengthens (Domjan, 2000, 2014). In contrast, there is evidence to show that simultaneous conditioning, where there is no delay between CS and US, is not effective (Lavond & Steinmetz, 2003). The study by Harvie et al. (2016), showed conditioning-induced hyperalgesia using simultaneous timing. Therefore, when considering classical conditioning in the context of pain, it is possible that any interval is less ecologically-valid than what actually occurs during the processing of nociception. That is, the nociceptive component of the compound stimulus (particularly input from the A delta nociceptors via their central projections) is not significantly delayed (Woolf, 2009). A trace interval similar to that used in Chapter 5 was also used in Chapter 7, which did show conditioning-induced hyperalgesia, suggesting that the difference may not have been a major factor in the conditioning effect. It is important to note that most conditioning studies that have investigated the effect of different trace intervals on learning have been undertaken in animals (Davis, Schlesinger, & Sorenson, 1989; Gallistel & Gibbon, 2000; Hoehler & Thompson, 1980), and that the modulatory effects of the trace interval vary depending on the type of response being recorded (i.e. automatic or subjective reporting; Gormezano et al., 1983; Lavond & Steinmetz, 2003).

A feature to consider when choosing the CS and US is the congruency between the two in relation to the type of response that is being elicited (Esber & Haselgrove, 2011; Haselgrove et al., 2010). While on the surface, the use of a right-hand image and painful stimulation to the right hand may seem highly congruent, there are in fact several potential problems. First, the features of the hand are not representative of the participant's own hand. This may have resulted in a disconnect between what the participant was observing and what their own hand looks like, perhaps preventing sufficient 'ownership' of the hand to induce a protective response (Ehrsson, Holmes, & Passingham, 2005; Hommel, 1997; Tsakiris, 2010). Second, the participant was observing an external image of a hand on a screen and feeling the stimulation of the painful laser on their right hand – creating a significant spatial discrepancy between the location of the conditioning stimulus and that of the noxious stimulus. This is not problematic for all conditioning studies—take Pavlov's dogs for example—but it may be problematic for the current thesis' objectives because it is a significant departure from what happens in reality—nociceptive and non-nociceptive

aspects are centred predominantly at the same location. One study (Harvie, Meulders et al., 2016), which showed conditioning-induced hyperalgesia, and another (Madden, Bellan et al., 2016), which showed conditioning-induced allodynia, used a CS that was spatially close to the US. Both studies used vibrotactile devices near the painful stimulation site. Conflicts between the visual features of the hand and the spatial difference between the CS (hand posture on a screen) and the US site (the back of the hand) may have contributed to the null findings of Chapter 5. Notably, however, the study reported in Chapter 7 and the studies by Jensen et al. (2012, 2014, 2015) also had this disconnect between the CS and US, yet they all demonstrated conditioning-induced hyperalgesia. The consistent aspect of all those studies was that they used faces as the conditioned stimulus, not hands or other body parts. Considering this evidence, it would therefore appear that face images are more effective CSs than hand images or other stimuli.

Why might face images be more effective than hand images at eliciting hyperalgesia? One possibility is the communicative value of faces. People are more accurate in reporting facial expressions than any other head or body movement (Knapp, Hall, & Horgan, 2013). The face can convey multiple emotions and can influence how others respond, such that a frightened face may induce fear in others (Karos et al., 2015). Moreover, the capacity of humans to recognise and discriminate between hundreds of different faces is unparalleled compared with their capacity to distinguish between other stimuli (Haxby, Hoffman, & Gobbini, 2000). This is thought to be due to the social importance of differentiating and recognising emotions from facial expressions (Karos et al., 2015). In the context of pain, people often express pain through facial expression, and different facial expressions may increase or decrease pain-related affect (Karos et al., 2015). Therefore, perhaps the circuitry is more easily recruited because of ‘inbuilt’ associative pathways (McNeil & Warrington, 1993). This pre-existing relationship between pain and faces may mean that the association between a painful stimulus and a face is easily accessible (LeResche & Dworkin, 1984; Williams, 2002). Therefore, it appears that the type of CS can have a hierarchical effect on the conditioned response. That is, the more ecologically-valid the stimulus is, the greater the elicited response (Domjan, 2005).

8.2.2 The response

A feature to consider when choosing the CS and US is the congruency between the two in relation to the type of response that is being measured (Esber & Haselgrove, 2011; Haselgrove et al., 2010). That is, the CS and US should match the anticipated response. For example, when investigating a taste-aversion response, a taste-based CS is more effective than an audio-visual CS. However, when investigating a fear response and using an electric shock as the US, audio-visual CSs are more effective than taste CSs (Domjan, 2005). This is thought to be because the audio-visual CS mimics the real world. Using an animal model as an example, mice are more likely to hear or see a snake before it attacks than to taste it (Öhman & Mineka, 2001). This has also been shown in pigeons, where an auditory CS is more effective than a visual CS when the US is an electric shock, but not if the US is food (Domjan, 2005). In studies of both monkeys and humans, CSs that are images of flowers and mushrooms are less effective for fear conditioning than for appetite conditioning (Öhman & Mineka, 2003). Therefore, it is important to ensure that if pain or hyperalgesia is the response to be assessed, the US and CS used are congruent.

However, perhaps the most congruent CS to use would be a movement or tactile input, because most persistent pain sufferers report pain on movement or from mechanical stimuli (Moseley & Flor, 2012; Vlaeyen, 2015). Movement was not used in this thesis because it comprises sensory, proprioceptive and motor outputs, and it is logistically very difficult to manipulate experimentally (Moseley et al., 2008). Therefore, movement has multiple variables which could confound the findings. Within fear conditioning studies, a joystick paradigm has been used to examine movement within a classical conditioning framework (Meulders & Vlaeyen, 2013b). That study investigated *fear* of movement-related pain as an outcome, rather than the pain itself, so it is problematic to extend that paradigm to the current question. The experiments in the current thesis were kept as simple as possible in order to manipulate only the independent variable. Tactile inputs have been investigated previously and have shown mixed results. Two studies investigating conditioning-induced allodynia used vibrotactile stimuli as conditioned stimuli (Madden, Bellan, et al., 2016; Madden, Russek, et al., 2016). While one demonstrated conditioning-induced allodynia (Madden, Bellan, et al., 2016), the other did not (Madden, Russek, et al., 2016). Another study of conditioning-induced hyperalgesia using vibrotactile stimuli as conditioned stimuli demonstrated a conditioned effect (Harvie, Meulders et al., 2016). This thesis used visual

images because some studies on pain expectancy and fear (Meulders et al., 2015) and conditioning-induced hyperalgesia (Jensen et al., 2014, 2015) have successfully used faces to demonstrate a conditioning effect.

8.2.3 The reinforcement schedule

The studies reported in Chapters 5 and 7 both used continuous reinforcement. This may have decreased the acquired salience of the CS, resulting in a decrease in the maintenance of the CR through the testing phases (Kaye & Pearce, 1984; Meulders et al., 2012; Rescorla & Wagner, 1972). In the psychology literature, it is largely accepted that predictability and uncertainty play key roles in determining stimulus salience (Esber & Haselgrove, 2011; Hogarth, Dickinson, Austin, Brown, & Duka, 2008; Wills, Lavric, Croft, & Hodgson, 2007). The question of *how* predictability and uncertainty influence stimulus salience is a cause for disagreement (Esber & Haselgrove, 2011; Haselgrove et al., 2010). Two theories exist that make opposite predictions (Haselgrove et al., 2010; Pearce, Kaye, & Hall, 1982). The first (Haselgrove et al., 2010) argues that stimuli that are good predictors of reinforcement (e.g. pain, danger or presence of food) will acquire high salience and increase stimulus attendance (i.e. continuous reinforcement), while stimuli that are poor predictors of reinforcement will lose salience and decrease attendance (i.e. partial reinforcement; Dunsmoor, Bandettini, & Knight, 2007; Esber & Haselgrove, 2011; Le Pelley, Oakeshott, Wills, & McLaren, 2005). The second theory (Pearce & Mackintosh, 2010) argues that stimuli that are good predictors of reinforcement lose salience because there is nothing further to learn from the association (i.e. continuous reinforcement), while stimuli that are poor predictors of reinforcement increase salience because further information is required regarding the stimuli and the possible consequences or reinforcement (i.e. partial reinforcement; Dunsmoor et al., 2007; Esber & Haselgrove, 2011; Pearce et al., 1982). The argument around partial versus continuous reinforcement is of particular importance to Chapters 5 and 7. Several studies (Esber & Haselgrove, 2011; Goosens, 2011; Meulders, Vansteenwegen, & Vlaeyen, 2012) have demonstrated that the conditioned response is better maintained during partial reinforcement than continuous reinforcement (Dunsmoor et al., 2007). This is thought to be due to the presence of an increased level of uncertainty, which maintains the salience of the stimulus. However, as the study in Chapter 5 did not show a conditioned effect and the study in Chapter 7 did, and both used continuous

reinforcement, the type of reinforcement does not appear to be the sole mechanism influencing the conditioned response.

8.2.4 The nature of the US

The type of modality chosen to reinforce the CS is important. While the choice of modality used in Chapter 5 differed to other studies which have demonstrated conditioned hyperalgesia (Harvie et al., 2016; Jenewein et al., 2013; Jensen et al., 2012, 2014, 2015), no other study has used a laser stimulus. The choice of modality used in Chapter 7 (electrocutaneous) was similar to that of two other studies which demonstrated conditioning-induced hyperalgesia (Arntz, 1991; Harvie et al., 2016). A possible explanation for the differences in the findings of the two thesis studies is that there were differences in the perceived averseness and arousal of the two modalities used (laser versus electrocutaneous). That is, laser preferentially, or perhaps solely, activates nociceptors in the skin (Plaghki & Mouraux, 2003), whereas electrocutaneous stimulation activates various non-nociceptive and nociceptive receptors. Therefore, the 'danger stimulus package' may have been quite different between the two modalities. Indeed, evidence has shown that modalities can differ in their established threat value (Coghill, McHaffie, & Yen, 2003; Tabor et al., 2013). From a functional perspective, individuals are often warned about the danger of electric shock and, therefore, the learnt association between electric shock and danger may already be established (Coghill et al., 2003). In contrast, a novel stimulus such as a laser may lack an established association and, therefore, participants' averseness and arousal to it could be lower (Tabor et al., 2017).

8.2.5 Distinguishability of the conditioning and generalisation stimuli

Chapters 4 and 6 each addressed the problem of perceptual discrimination between the CSs and GSs that were subsequently used in Chapters 5 and 7. Chapter 4 addressed the issue of differences between stimuli, and their categorisation, being manipulated and sequenced based on researchers' perceptions, rather than those of participants. This risks participants' responses being analysed according to researchers' categorisation and sequencing of stimuli. Failure to investigate differences in the sequencing of stimuli could result in the incorrect interpretation of the results of generalisation studies (Struyf et al., 2015). Subsequently, the results may not represent differences in precision and generalisation, but differences in perceptions of how stimuli should be sequenced. Therefore, Chapter 4 was essential for determining whether the researchers' strategy for sequencing the proposed stimuli (to

subsequently be used in Chapter 5) matched that of the majority of respondents. This instilled confidence that any differences in the Chapter 5 responses were due to generalisation, rather than differences in sequencing of stimuli and stimulus categorisation.

Chapter 6 investigated the perceptual discrimination of the face image stimuli. Stimulus generalisation studies assume that participants discriminate between a conditioned stimulus (CS) previously associated with an unconditioned stimulus (US), and a novel but similar stimulus (GS). That is, the participant perceives that the CS and GS are different. However, the GS has similar characteristics to the CS and, therefore, the meaning attached to the CS is extrapolated to the GS, resulting in a similar CR being elicited by both (Vervliet & Van, 2015). It was discovered in Chapter 6 that respondents were unable to distinguish between the most similar faces, prompting further refinement of the stimuli. Failure to have addressed this problem in Chapter 6 would have affected the interpretation of the Chapter 7 results. As it was highly likely that participants could not distinguish between the similar faces in Chapter 7, the CR was, therefore, just a CR, and not a generalised CR (Struyf et al., 2015).

8.3 Limitations

Several limitations of this thesis need to be considered when interpreting the results. The most notable is the issue of whether novel stimuli elicit a conditioned response because they are very similar to the conditioned stimulus, or because they cannot be distinguished from it (Moseley & Vlaeyen, 2015; Struyf et al., 2015). The former explanation suggests that overgeneralisation of a pain response could be due to imprecise encoding and subsequent cortical representation of the painful event (Moseley & Vlaeyen, 2015). The latter suggests that novel stimuli are perceived to be the same as the CS because, they are indistinguishable from it (Struyf et al., 2015). Both arguments are equally plausible, and in fact they are not necessarily mutually exclusive. However, neither can be validated until assessments of perceptual discrimination, which are independent of assessments of stimulus generalisation, are undertaken. Independent assessment of perceptual discrimination and generalisation would provide insight into whether the observation is one of a deficit in perceptual discrimination and/or an overgeneralisation of the response (Struyf et al., 2015; Vervliet & Van, 2015). This issue is not simply semantic; in order to investigate the imprecision hypothesis, it is necessary to first disentangle the roles of perceptual discrimination and generalisation in pain (Struyf et al., 2015).

The necessity to break down the imprecision hypothesis (Moseley & Vlaeyen, 2015) and test each individual assumption experimentally may have left it too far removed to accurately reflect the real world. This meant that certain experimental approaches within this thesis had limited ecologically validity. First, the experimental settings used to investigate pain were removed from the real world. Therefore, perhaps it does not matter which stimulus modality researchers use to elicit pain (Eccleston & Crombez, 1999), as experimentally-elicited pain is less salient than clinical pain (Somogyi, Tamas, Lujan, & Buhl, 1998; Zetzsche, 2005). Second, images that are not of participants' own hands or faces may be less salient than ones that are. This could be tested using individualised images for each patient. While this might better assess individual responses, it would be logistically challenging and could not be generalised beyond the individual. This strategy also brings in other perceptual problems, such as how one may view him/herself, or whether they like to look at images of themselves (Kim, Oh, Chiaburu, & Brown, 2012). This could result in a modulation of valence, which itself may impact on the overall pain experience (Kunz et al., 2012).

A further limitation of this thesis stems from the issue of habituation to noxious stimuli (McMahon, Koltzenburg, Tracey, & Turk, 2013). Literature on non-associative pain has shown that repeated exposure to a noxious stimulus can cause reported pain levels to gradually decrease (McMahon et al., 2013). The arousal level of the participant has been identified as an influence of how long it takes for habituation to occur (McMahon et al., 2013). Certainly, in classical conditioning experiments conducted over long time periods, the arousal level of participants varies. However, one could assume that it decreases over time, particularly when so many aspects of the experiment are controlled. Lighting, ambient noise level and temperature of the experiment room are controlled to minimise distractions. These factors could contribute to low participant arousal which, in effect, promotes habituation and could alter the preceived intensities of the experimental stimuli and thus the learning process. Previous studies have grappled with this issue of habituation (Harvie et al., 2016; Madden, Bellan, et al., 2016; Madden, Harvie, et al., 2016; Madden, Russek, et al., 2016) and a clear solution remains elusive.

The variability of pain and the difficulty of assessing it is a potential limitation of this thesis, as it is for all pain research (McMahon et al., 2013). The methods used to assess pain relied on three fundamental assumptions. The first assumption is that participants are able to accurately report their pain on a single subjective rating scale (McMahon et al., 2013). The second assumption is that participants can reliably rate the magnitude of their pain (McMahon et al., 2013). The third assumption is that participants can quickly and accurately change their responses between ratings of expectancy of a painful stimulus and ratings of how painful it was (Price & Harkins, 1992b). These assumptions make the study of pain difficult because use of subjective ratings is the most common way of assessing pain. However, potential solutions to these problems can introduce other problems. For example, if an excessively detailed description about the rating scale is given, it may increase the reporting bias of participants (Price & Harkins, 1992b) and make it difficult to pool data from multiple participants. Another potential solution is to use multiple measures of pain, such as ratings of pain intensity and unpleasantness (Price, 1999). However, it has been shown that these ratings are subject to reporting bias, and measuring them separately provides no more information than if a single pain measure was used (Chapman et al., 2001, 2002). One potential method to address some of these assessment-related limitations is to include a ‘first person’ perspective (Thacker & Moseley, 2012). This approach requires qualitative

analysis, but might yield powerful insights that could then be investigated using quantitative approaches.

Some specific limitations of the studies presented in this thesis are as follows: First, Chapters 4 and 6 investigated different components of perceptual discrimination. Chapter 4 investigated whether other individuals sequenced eight hand images in the same way as the researchers did. Chapter 6 investigated whether individuals could distinguish between face stimuli. However, it would have strengthened both chapters to determine the distinguishability of the hand posture images in Chapter 4 and the sequencing of the face stimuli in Chapter 6. Second, Chapters 5 and 7 used different questionnaires to evaluate different affective states. It would have been more advantageous to have used the same questionnaires for both studies. This would have enabled pooling of the data and allowed a more rigorous investigation into the roles that different affective states play in the extent of generalisation of conditioning-induced hyperalgesia. Third, the period of non-reinforcement and pre-exposure of the GS in Chapter 5 may have contributed to the null findings, and therefore is a limitation of the study. That is, in Chapter 5, participants were pre-exposed to the GS during the testing of US expectancy, and not one of the GSs were reinforced at that time. Therefore, the classical conditioning effect could have been extinguished before the next testing phase, when generalisation of conditioning-induced hyperalgesia was tested. Certainly, the lack of reinforcement before the testing of generalisation of conditioning-induced hyperalgesia may play a role, because in Chapter 7, after the first test phase, participants went through a Re-acquisition phase before the testing for generalisation of conditioning-induced hyperalgesia began.

8.4 Clinical implications

If persistent pain develops due to an imprecise encoding and representation in the brain of the original painful event, and if this results in responses generalising to stimuli that are less and less similar to the original event, it is reasonable to assume that treatments which reduce imprecise encoding might be effective in limiting the development of chronic problems. First, in a clinical context, the finding that a lack of contingency awareness may influence the generalisation of US expectancy, highlights the need to ensure that pain sufferers are aware of the exact stimuli (e.g. movement or position) that evoke their pain and perhaps, more importantly, the ones that do not (Craske & Mystkowski, 2006; Hofmann, 2008). This opens a pathway towards incorporating extinction-like practices when dealing with people who have acute pain. By limiting the potential for patients to respond to less similar GSs or 'false alarms', the association between these GSs and the response could be extinguished (Craske & Mystkowski, 2006; Meulders, Meulders, & Vlaeyen, 2014). This extinction-like practice causes the less similar stimuli to lose its predictive value of the US ultimately leading to a more precise representation of the CS and therefore a precise response (Hofmann, 2008; Vlaeyen, 2015).

Second, the issue of perceptual discrimination and generalisation is relevant not only to pain, but also to a number of other persistent conditions (Dymond et al., 2015; Struyf et al., 2015; Vervliet & Van, 2015). That is, generalisation is thought to be a fundamental process involved in other persistent conditions such as generalised anxiety disorder (Dymond et al., 2015; Lissek et al., 2008). Multiple factors can make-up fear generalisation and, as such, a difference in generalisation between healthy controls and persistent pain or anxiety populations may be related to any one of those factors (Lissek et al., 2008). That is, generalisation and perceptual discrimination may not be opposites, but both may interact and contribute to the generalisation of pain or anxiety (Struyf et al., 2015). For example, clinically, two patients may have similar presentations that indicate generalisation to similar stimuli. In the first patient's case, the major contributing factor may be their inability to distinguish between dangerous stimuli and other stimuli. That is, more stimuli (sensory inputs and contextual cues) are perceived to be the same as the initial compound pain stimulus (Struyf et al., 2015). Therefore, for this patient, training which improves perceptual discrimination may help to reduce pain. However, the second patient may be able to

distinguish between the different stimuli, but has extrapolated dangerous meanings to stimuli which are not dangerous (i.e. overgeneralised; Struyf et al., 2015). In this case, treatments such as exposure therapy or cognitive behavioural therapy (CBT) may reduce excessive extrapolation and therefore pain. This explanation, albeit somewhat speculative, may explain *some* of the disparity between studies examining the effect of tactile acuity training in pain sufferers (training to distinguish between two tactile stimuli; Catley, O'Connell, Berryman, Ayhan, & Moseley, 2014; Eads, Moseley, & Hillier, 2015; Grushka, Sessle, & Howley, 1987; Luomajoki & Moseley, 2011; Stanton et al., 2013).

Third, the ability to modulate the expectancy of a painful stimulus through conditioning and generalisation highlights that individuals' expectations may influence their responses. This is relevant to the fear-avoidance model, because expectancies have been shown to be modifiable and, therefore, addressing pain expectancy could reduce pain (Vlaeyen & Linton, 2012). This could also be relevant for the Bayesian model of pain perception that stipulates that expectancies influence perceptual processing (Tabor et al., 2017). Therefore, addressing pain expectancies could alter perceptual processing and reduce, rather than enhance, pain. Third, although more evidence is required, treatments that improve perceptual discrimination, such as tactile discrimination training, have been shown to reduce pain (Luomajoki & Moseley, 2011; Moseley, Zalucki, & Wiech, 2008; Stanton et al., 2013).

8.5 Contributions of this thesis to the field

This thesis has improved understanding of some of the assumptions of the imprecision hypothesis. Therefore, a few provisional conclusions are made regarding the imprecision hypothesis:

(1) Healthy participants showed generalisation of conditioning-induced hyperalgesia to similar face stimuli.

(2) The less aware participants are of the contingency between the CS and US, the more imprecise their expectancy response to the painful US will be.

(3) Low positive affect and high negative affect states may be associated with increased generalisation of conditioning-induced hyperalgesia.

Moreover, this thesis also highlights several considerations for the design of classical conditioning studies investigating the assumptions of the imprecision hypothesis:

(1) Further research is required to determine the best cognitive manipulation for selectively modulating the sensory and affective dimensions of pain.

(2) The distinguishability of the conditioning and generalisation stimuli, and the sequencing of the conditioning and generalisation stimuli, are important considerations when interpreting the results of generalisation studies.

(3) The stimulus, response and reinforcer are important determinants of conditioning effects.

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

9 References

Publications, presentations and awards

Statements of authorship

Appendices

9. References

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Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

10 Publications, presentations and awards

Statements of authorship

Appendices

10. Publications, presentations and awards

10.1 Publications throughout candidature

Wallwork, S. B., **Talbot, K**., Camfferman, D., Moseley, G. L., & Iannetti, G. (2016). The blink reflex magnitude is continuously adjusted according to both current and predicted stimulus position with respect to the face. *Cortex*, *81*, 168-175.

10.2 Presentations throughout candidature

10.2.1. Invited conference presentations

Talbot, K. A. (2016). *Pain in podiatry*. Paper presented at the South Australian State Podiatry Conference, South Australia, Australia.

Talbot, K. A. (2016). *The effect of affect*. Paper presented at Women in Science, University of South Australia, Australia.

10.2.2 Invited talks

Invited to speak on Insight SBS, Episode 3, *Placebo* 2016

10.2.3 Invited Guest lectures

Talbot, K. A. (2016). '*Neuropathic pain*', Podiatry Department, University of South Australia.

Talbot, K. A. (2016). '*The effects of verbal communication on pain*', Podiatry Department, University of South Australia.

Talbot, K. A. (2016). '*Pain and classical conditioning*', Podiatry Department, University of South Australia.

10.3 Scholastic activities during candidature

Explain Pain Course, Melbourne, Australia 2015

Medical Neuroscience online course, Duke University, North Carolina

Women and Children Hospital, Research Project, Adelaide, Australia

10.4 Community engagement

Advisory scientific member for '*Pain Free*' 2016 State Podiatry Conference, South Australia, Australia.

Volunteer at the Ride for Pain 2014, 2015

10.5 Peer-reviewed poster presentations

Kerwin A. Talbot, Victoria J. Madden, Sara Jones, G. Lorimer Moseley 2016, '*Pain's sensory and affective qualities – differentially modifiable dimensions or two aspects of a unitary experience? A Systematic review*.' International Association for the Study of Pain, Yokohama, Japan.

Kerwin A. Talbot, Victoria J. Madden, Fredric Tebmer, Daniel S. Harvie, Valeria Bellan, Sara Jones, G. Lorimer Moseley, 2017, ' *Do healthy controls generalise conditioned hyperalgesia to different hand postures*', Australasian Podiatry conference, Melbourne, Australia.

10.6 Grants and awards throughout candidature

Australian Government Research Training Program 2014

Pain Adelaide Poster prize (first prize) 2016.
The University of South Australia, Health Science, Research Week Poster Award (runner up) 2015.
The University of South Australia, Health Science, Conference Scholarship 2016.

Introduction

Methods

Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review.

Placing stimuli in order: Conflicts between the choices of researchers and participants.

Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?

Are these faces different? Perceptual acuity for different faces – an electronic survey.

Healthy controls show conditioned generalisation of hyperalgesia to face images.

Thesis discussion

References

Publications, presentations and awards

11 Statements of authorship

Appendices

Statement of authorship for manuscript entitled **"Pain's sensory and affective qualities – differentially-modifiable dimensions or two aspects of a unitary experience? A systematic review".** This article is under review at the Clinical Psychology Review, at this stage has not been accepted for publication and thus permission to include this article was not required.

Authors: Kerwin Ashleigh Talbot[a], Victoria J. Madden [a]; Sara Jones [a] G. Lorimer Moseley*[a]

Affiliations

[a] Sansom Institute for Health Research, University of South Australia, Adelaide, Australia

Name of candidate: Kerwin Talbot

Study conception and design; data collection, extraction and analysis; manuscript preparation; journal revisions.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed: [redacted]

Date: 11/07/2017

Author 2: Victoria J. Madden
Study concept and design; data collection; interpretation; manuscript preparation.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed:

[redacted]

Date: 07/07/2017

Author 3: Sara Jones
Study interpretation and manuscript preparation.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed:

[redacted]

Date: 10/07/2017

Author 4: Lorimer Moseley
Study conception and design; interpretation; manuscript preparation.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed:



Date: 10/07/2017

Statement of authorship for manuscript entitled **"Placing stimuli in order: Conflicts between the choices of researchers and participants"**. This article is not currently under review and therefore permission to include this article was not required.

Authors: Kerwin Ashleigh Talbot[a], Victoria J. Madden [a,b], Sara Jones [a] G. Lorimer Moseley[a]

Affiliations:

[a] Sansom Institute for Health Research, University of South Australia, Adelaide, Australia

[b]Department of Anaesthesia and Perioperative Medicine, Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa.

Name of candidate: Kerwin Talbot

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Statement of authorship for manuscript entitled **"Do healthy controls generalise conditioned hyperalgesia to similar but novel stimuli?".** This article is not currently under review and therefore permission to include this article was not required.

Authors: Kerwin Talbot[a], Victoria J Madden [a,b], Fredric TeBmer[a], Daniel S Harvie, Valeria Bellan[a], Sara Jones[a], G. Lorimer Moseley[a]

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Study interpretation and manuscript preparation.

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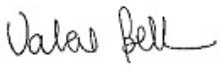
Signed:


Date: 08/07/2017

Author 4: Valeria Bellan
Study interpretation; assisted with data collection; manuscript preparation.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed:



Date: 10/07/2017

Author 5: Sara Jones
Study interpretation and manuscript preparation.

I hereby certify that this statement of contribution to the above-named paper is correct.

Signed:

Date: 10/07/2017

Author 6: Lorimer Moseley
Study conception and design; interpretation; manuscript preparation.

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Signed:



Date: 10/07/2017

Statement of authorship for manuscript entitled **"Are these faces different? Perceptual acuity for different faces – an electronic survey"**. This article is not currently under review and therefore permission to include this article was not required.

Authors: Kerwin Talbot[a], Victoria J Madden[a,b], Sara Jones[a], G. Lorimer Moseley[a]

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Date: 10/07/2017

Statement of authorship for manuscript entitled **"Healthy controls show conditioned generalisation of hyperalgesia to face images"**. This article is not currently under review and therefore permission to include this article was not required.

Authors: Kerwin Ashleigh Talbot[a], Victoria J. Madden[a,b];Daniel S. Harvie[a], Sara Jones[a], G. Lorimer Moseley[a]

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12. Appendices

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12.1 Appendix 1 Chapter 3 systematic review protocol

Working title of study: Can the sensory and affective dimensions of pain be selective modulation? A systematic review

Kerwin A Talbot[1] , Victoria J Madden[2] ,Sara Jones[3], G Lorimer Moseley[4]

Protocol description

This is a protocol for a review. The primary objective is as follows: To determine the extent to which the affective and sensory dimensions of pain can be selectively and intentionally modulated by different psychological techniques, in both chronic pain and healthy populations.

Introduction

Pain evolution

The conceptualisation of pain has evolved from a linear relationship, where pain was merely an output generated due to tissue injury, to a dynamic and complex experience that involves a multitude of varying processes. Through research it has become abundantly clear that pain can be experienced without the presence of tissue injury, or that tissue injury can exist without the experience of pain.

A multidimensional understanding

Through the progression of the understanding of the pain experience, research has identified that pain has multiple dimensional inputs; that pain is not solely a sensation but has an affective dimension as well.

As early as 1900, Sherrington proposed that all sensations had a corresponding affective quality. This concept was continued by Hardy, Wolff, and Goodell (1952), who identified that pain consists of both a sensation, and a reaction or processing component. Although it is widely recognised that pain consists of both a sensory dimension and an affective dimension, how much these two dimensions can be separated is debated among researchers.

Why this review is important

Whilst it is clear that multiple dimensions of pain may co-exist, it is not known whether these dimensions are processed simultaneously (in parallel) or one after another. Furthermore, are cortical areas different, or are the dimensions of pain too closely intertwined to warrant separation?

Objectives

The primary objective is to determine the extent to which the affective and sensory dimensions of pain can be selectively and intentionally modulated by different psychological techniques, in both the chronic pain and healthy populations.
This primary objective was broken down into four key areas that the review attempted to examine.

1. Can the sensory and affective dimensions of pain be selectively and intentionally modulated?
2. To what extent can the sensory and affective dimensions of pain be separately analysed?
3. What is the efficacy of different psychological tools in selectively modulating the two dimensions?
4. Is there a difference between chronic pain and healthy populations?

Methods

The criteria that will be used when considering studies for this review are as follows.

Type of studies

This review will include experimental studies that examine the efficacy of a psychological technique in modulating the sensory and affective dimensions of pain. Studies will be required to have the title and abstract in English to ascertain their eligibility. Any eligible non-English full-text studies will be translated by two independent translators. The content will then be independently assessed by the two reviewers (KT & VM) to determine its suitability for inclusion in the study.

Types of participants

Studies that included either chronic pain or healthy participants will be eligible for this review. Studies which examine chronic pain participants will need to have identified a diagnosis of stable pain for longer than 3 months to be considered for this review. There is no age limit set for the search, and studies that are found to fit all the inclusion criteria will be included.

Types of interventions

Studies that used a psychological technique to intentionally modulate either the sensory or affective dimension of a participant's pain perception will be included. Studies which investigate somatosensory (i.e. TENS) or exogenous pharmacological effects on pain perception will be excluded.

Types of outcome measures

Primary outcomes

Studies must have the following to be eligible for inclusion:

1. Measure both sensory (intensity) and affective (pleasantness) dimensions of pain using a valid outcome measure.
2. The tool used to measure the dimensions must have clearly defined anchors, if the anchors are not clearly defined then the script will be sought for clarification.
3. If neither anchors nor the script are available, then the authors will be contacted with questions relating to the nature of their outcome measures and clarification on the script.
4. Studies must measure the sensory and affective dimensions of pain both before and after the experimental psychological technique has been implemented. If no baseline measure was provided with regards to the affective and sensory nature of the participants, then this aspect will be assessed in the Risk-of-Bias form, accordingly.

Primary outcomes, if similar, will be presented and analysed as changes on a continuous scale or in a dichotomised format. Clinical importance will be analysed according to the effect size and will be judged in reference to the IMMPACT consensus statement.

Secondary outcomes

Secondary outcome measures, when available, will be analysed as follows:

1. Cortical imaging of alterations in brain activation during the processing of pain during different psychological manipulations such as fMRI and PET.
2. Any data obtained that measures psychological variables which would potentially modulate the affective component of pain, i.e. personality traits, anxiety levels.

Secondary outcomes will be presented narratively and any differences or similarities will be highlighted. However, it is not the main aim of this review to quantify the changes in cortical processing among the different included articles.

Search methods for identification of studies

Electronic searches

Selected databases will be searched and stringent keywords applied to enable collection of all the relevant and available literature. The following databases will be used:

- Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library
- MEDLINE (OVID)
- SciVerse SCOPUS
- PsycINFO (OVID)
- EMBASE classic & EMBASE

The OVID MEDLINE initial search strategy will be developed and modified. All preceding database searches will follow this outline. If required, adaption to individual databases will occur.

Searching other resources

Pearling

All eligible studies, key textbooks and previous systematic reviews references will be searched to ensure all the literature pertaining to this topic is included.

Unpublished data

Unpublished data will be sought to minimise publication bias, through requests from key authors for any studies pertaining to the question. As well as asking for any other literature by other researchers that they are aware of that would be crucial to include in this review.

Data collection and analysis

Selection of studies

The title and abstracts of studies identified by the search strategy will be exported into Endnote. The studies will be independently evaluated by two reviewers (KT & VM) and will be excluded if they fail to meet the outlined criteria. Any disagreements will be resolved through discussion. If a resolution cannot be reached, then a third reviewer (GLM) will assess and a majority decision will be made.

Eligible studies' full texts will be evaluated independently by both reviewers using a standardised inclusion and exclusion checklist. Throughout this process, duplicates and reasons for excluding studies will be recorded in an Excel spreadsheet to allow for accurate reporting.

Data extraction and management

Reviewers (KT & VJM) will independently extract data from included studies and record the data on a standardised form. Any discrepancies will be discussed and if a resolution cannot be found, a third reviewer will be included. The third reviewer (GLM) will assess the particular study and a majority decision will be made. The following data of interest will be extracted:

- Primary aim(s)
- Participant data (baseline characteristics)
  - Age
  - Gender
  - Ethnicity
  - Scales of suitability
- Study intervention basics
  - Inclusion criteria
  - Exclusion criteria
  - Recruitment method
  - Source of participants
- Trial characteristics
  - Study design
  - Experimental / treatment group
  - Control group

- Sample size data
- Outcomes
  - Primary outcomes measures
  - Secondary outcome measures
- Statistical analysis
  - Mean/median
  - SD/IOR
  - Effect size
  - CI

If there is more than one psychological technique used, then the data will be extracted from both. The data from the first phase of the cross-over trial will be analysed for meta-analysis. If anchors used in the assessment of participants' perceived pain rate the stimulus, the data that is collected will then be dependent on whether the study had a calibration phase. If no calibration was conducted, then only the data pertaining to participants that rate the stimulus as painful will be extracted.

If further detail is required, then the authors will be contacted electronically. Open-ended questions regarding the information required will be used to prevent the likelihood of overly positive answers. A maximum of six weeks will be given for the author to reply, reminder emails will be sent after two and four weeks to encourage a response. If no response is received then, only the usable data fom the article will be extracted and analysed.

Assessment of risk-of-bias and the quality of selected studies

Each included study's risk of bias will be analysed using an adapted Cochrane 'risk of bias' assessment tool, and will be given a classification of either low, high or unclear, as outlined in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. Risk-of-bias pertaining to specific experimental design issues will be considered and assessed according to the following criteria:

- Sampling and recruitment
- Blinding
- Psychological technique

- Primary outcome measurement
- Secondary outcome measurement
- Containments
- Reporting
- Baseline characteristics
- Appropriateness of statistical analysis

Certain criteria were determined to be important in relation to the review question and therefore had a higher weighting when determining the risk of bias. If further information was required to make a decision regarding the level of bias, then the authors were contacted and asked for additional information. The same protocol was followed as that outlined for data extraction. If the authors failed to respond, the study's bias will be rated according to the level of information provided in the article.

Data synthesis

If sufficient data is available, then the results will be pooled using the report difference in sensory and affective pain ratings. The data will be collected and grouped into primary and secondary outcomes. A meta-analysis will only be conducted if the data is sufficiently homogenous.

Representation of the outcomes for the 'risk of bias' of included studies will be clearly shown. If statistical pooling is ill-advised due to insufficient data, then a narrative synthesis of the evidence will be performed.

Measures of treatment effect

The effect size for each individual component of pain (intensity and unpleasantness), as measured with a VAS or NRS, will be conveyed separately using the mean difference (if the same measurement scale was used) or the standardised mean difference (if different scales are used).

Assessment of heterogeneity

Studies with similar outcome measures and psychological techniques, with importance given to the analysis of affective versus sensory ratings of pain, will be pooled. Affective ratings will not be pooled with sensory ratings and two different psychological techniques will not be grouped.

Chi-squared tests will examine the statistics for heterogeneity; the threshold for the interpretation of I^2 will be 0—60% where:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity

If the heterogeneity is in the high range (70% or higher), then the subgroups will be analysed for heterogeneity.

Subgroup analysis and investigation of heterogeneity

If heterogeneity is high, then subgroups will be identified and analysed accordingly. However, if subgroups are not feasible, then a narrative synthesis will be used.

Assessment of reporting biases

During the meta-analysis, if small study effects and publication bias are identified as a source of exaggerated effect size, then appropriate statistical analysis will be completed to explore this issue. One potential method to analyse this is with funnel plots.

Sensitivity analyses

To determine the robustness of our findings, a sensitivity analysis will be completed. To determine the sensitivity of this review, a re-analysis of the data using a fixed effects model will be conducted.

Narrative synthesis

If the studies collated are only loosely related, it would be inappropriate to perform a meta-analysis. Then, a synthesis considering the quality of the studies and limitations of the overall evidence base will be completed.

12.2 Appendix 2. Chapter 4 survey protocol

Working title of study: How do people sequence hand images? E-survey

Kerwin A Talbot[1], Victoria J Madden[2], Sara Jones[3], G. Lorimer Moseley[4]

Protocol description

This is a protocol for a survey. The primary objective of the survey is as follows: To determine whether people intuitively sequence eight hand images the same way as the researchers have sequenced them.

Introduction

Generalisation of novel but similar stimuli has been shown in many conditions, such as post-traumatic stress syndrome. Hypervigilance for stimuli that relate to an initial inciting event may occur through various cognitive mechanisms. Previous Pavlovian experiments investigating the generalisation of imagined painful hand postures to novel but similar stimuli have demonstrated that pain-related fear spreads to other similar but distinct hand postures along a generalisation gradient. This gives rise to a central question: Can we assume that, within a population, everyone's perception of what constitutes a similar visual stimulus is the same? Bayesian theory suggests that a person's perception is composed of current evidence integrated with past experience. With this in mind, do past experiences, prior knowledge such as hand pain, and other factors influence a person's perception of novel but similar stimuli? Would this altered perception alter their generalisation gradient? Finally, does the manner in which one sequences stimuli remain stable?

Aims

This study has two aims. First, to investigate the reliability and validity of the order of a chosen experimental hand sequence with the general population, and second, to investigate whether there are any trends that exist in the chosen sequence that relate to previous pain experience, demographics and hand pain and disability.

Hypotheses

That the majority of people sequence pictures of hands according to a functional postural progression.

That a history of hand pain or disability results in deviation from the usual sequence.

Ethics

The introductory page is a brief synopsis of what the survey is about. The volunteer is then asked to consent to completing the survey. If they tick 'I agree', then the survey will allow

them to continue on. However, if they tick ‘I disagree’, the survey will automatically close. An amendment for ethics approval will be sought.

Methods

Sample size

100 participants.

Recruitment

An online survey will be made available through social media and word-of-mouth to adults (> 18 years). Volunteers will be asked to answer 18 questions relating to demographic characteristics, occupation-related variables and pain-related experiences.

Piloting and development

The survey will be created and individually drafted by four assessors (KT, SJ, and VJM and GLM). The main focus of the drafting process will be to ensure the clarity of the questions. The survey will then be piloted on a larger group of people, and their comments and feedback will be evaluated and incorporated into the survey as appropriate.

The survey will be administered to the respondent population and data will be collected and transferred into Excel. The survey will be open for a total of six weeks. If participants agree to be contacted again, a second survey will be sent out to these participants approximately three weeks after completing the initial survey. Consenting participants will have a total of two weeks to respond to the second survey, after which they will be considered a non-responder. The surveys will be deleted following extraction and analysis of all the data. Data will only be used for research purposes. All data that is collected for the first survey will be anonymous, unless participants provide the researchers with their contact details for follow-up. The details provided will then be kept confidential and secure. On completion of the follow-up process the participants’ contact details will be deleted.

Survey questions

Three questions address demographics, with a specific question relating to profession. Two questions address chronic pain and nine questions address hand pain / injury and disability associated with hand pain. The remaining two questions ask participants to order selected hand images into 'a logical sequence' and to provide an explanation for the chosen sequence. The images are comprised of eight different hand postures ranging from fully flexed to fully extended. The pain-related questions will be randomised to either appear before the sequencing question or afterwards.

Analysis

Questions 1 to 15: Responses will be recorded and represented graphically as percentiles.

Questions 16 & 17: Responses will be entered into SPSS. Sequences that match the predicted sequence will be assigned a 'yes' and those that do not will be assigned a 'no'. A logistical regression analysis will be performed using SPSS. A univariate analysis will be performed using the 'correct response' as our dependent variable and each of the following mentioned below as the independent variables. Significant variants will then be entered and a multivariate analysis will be performed to determine the influence of each independent variant.

The following variables will be considered during the analysis:

- Pain anywhere other than in the hand
- Age
- Gender
- Being pain-free
- Current hand pain (this will also include wrist and forearm pain)
- Hand arthritis (this is a separate group as people may have arthritis without pain)

Question 18: will be used to determine whether the participant will be sent a follow-up survey or not, on the grounds of whether they consent to be contacted again. Once again, a logistical regression analysis will be performed to determine whether their initial sequence matches their secondary sequence.

*Any surveys that have missing sequence numbers will be excluded from this analysis.

12.3 Appendix 3. Chapter 5 experimental protocol 1

Working title of study: The generalisation of expected pain using classical conditioning in healthy controls.

Kerwin A Talbot[1] , Victoria J Madden[2] , Daniel S Harvie[3], Valeria Bellan[4], Sara Jones[5], G Lorimer Moseley[6]

Protocol description

This is a protocol for an experiment. The primary objective is as follows: To determine the extent of generalisation of expected pain in healthy controls.

Introduction

A study that investigated motor imagery and pain-related fear demonstrated that imagined movements can elicit pain-related fear (Meulders et al. 2015). Using a classical conditioning paradigm, the study taught participants to associate one imagined movement (conditioned stimulus, CS) with a painful stimulus (unconditioned stimulus, US) and recorded the participant's predicted fear of the painful imagined movement (conditioned response, CR). The findings demonstrated that, 1) ratings of predicted fear were greater for the imagined movement associated with the painful stimulus than for the movements that were not, and 2) the predicted fear extended to similar but distinct imagined movements (generalisation). Clinical observations suggest that pain is influenced by contextual factors (Karos, Meulders & Vlaeyen, 2015). That is, pain can be influenced by a range of different stimuli, such as a particular image, thought, feeling-state, or person, and not solely by movement (Gatchel & Turk, 1999; Loggia, Mogil & Bushnell, 2008; Meulders & Vlaeyen, 2013; Price, Harkins & Baker, 1987; Walco, 2008). This study will use a classical conditioning paradigm to examine whether the predicted likelihood of pain (CR) associated with particular images of a particular hand posture (CS+) is generalised to images of novel but similar hand postures.

Aim

1. To determine the extent of generalisation of predicted pain experience (CR) to hand posture images that differ from the hand posture image (CS+) that was originally paired with a painful US.

Objectives

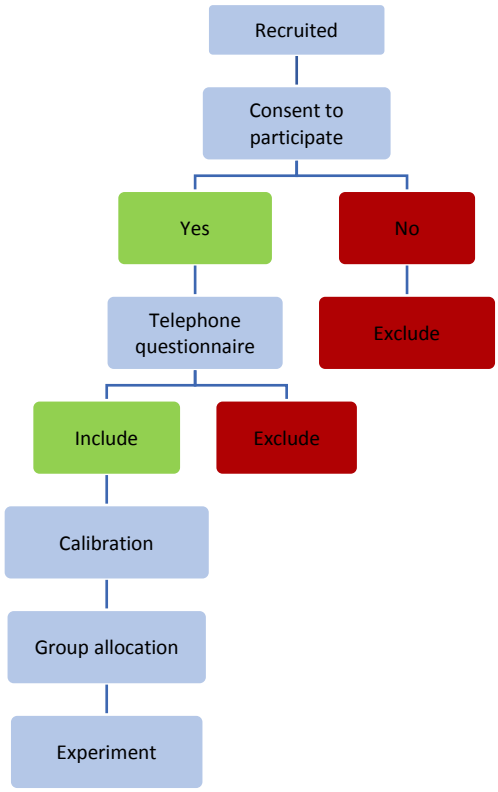
1: To investigate the predicted likelihood of pain to images of hand postures (generalisation stimuli, GSs) that are of graded similarity to the originally painful hand posture (CS+).

2: To investigate whether the predicted likelihood of pain associated with the CS+ and CS- postures modulates the generalisation of predicted likelihood of pain to hand postures of varying similarity to the originally painful hand posture (CS+).

3: To investigate whether contingency awareness is associated with the difference in predicted likelihood of pain associated with the CS+ and CS-.

4: To investigate whether the strength of the differential conditioned response is related to contextual fear ratings (Ploghaus et al. 2003).

Overview



Recruitment

Based on a previous study with similar methods, sample size calculation was completed *a priori* using G*power (Faul, Erdfelder, Buchner & Lang; Institute of Psychology, University of Duesseldorf, Version 2, Germany) on a Windows computer. To detect an effect size of 0.4, with a power of 80% and alpha of 0.05, it was calculated that this study needs to recruit 16 participants.

All potential participants will be contacted and if they are interested, an information sheet will be emailed to them. If they are willing to participate, the participant will be asked several questions about their general health. The answers will determine their eligibility to participate in the study. The following exclusion criteria will be assessed:

- Neurological diseases
- Current chronic pain (pain which has lasted longer than 12 weeks and is present on most days (Green 2013))
- Previous, recent history of chronic pain (within the last 6 months)

- Pregnancy status
- Current pain (at any site)
- Hand pain or recent hand injury (unless they have had complete resolution and no symptoms for 12 weeks)
- Inability to communicate effectively using English (reading and speech)

The following safety criteria will be assessed to identify any additional risk associated with the use of a Nd:YAP laser.

Exclusion criteria related to use of the laser:

- Minor or major illnesses – a list of all common illnesses that would alter heat or cold sensitivity was compiled (Attachment One).
- Medications that alter heat and cold sensitivity (e.g. opioids) - a list of common medications that would alter thermal sensitivity was compiled (Attachment One).
- Altered skin sensation (e.g. peripheral neuropathy of the hand, significant scars or burns, Raynaud's disease)
- Psychiatric illnesses (e.g. schizophrenia, bipolar disorder)
- Skin conditions on the upper limb and hand (e.g. eczema)

If the participant meets one or more of the above criteria, then they will be excluded from the study. It will be more time and cost effective, for both the participant and experimenter, if participants are screened via the telephone. If eligible, a time will be arranged for participants to come in to the testing location and complete the participant informed consent process. After informed consent is obtained, the participant will be re-screened to check their eligibility for inclusion. Volunteers who have recently participated in conditioning experiments will be excluded, except if: it has been more than six months since previous participation, or the nature of the conditioning experiment was very different to the current experiment (i.e. different stimulus and different delivery of stimulus).

Methods

Materials and measures

Conditioning stimuli: images of certain hand postures.

Unconditioned stimulus: Nd:YAP laser.

Images of different hand postures will be used as the conditioned stimuli (CSs). The CS+ and CS- will be assigned to two specific hand postures, and the participant will view four different orientations of the CS+ and the CS-, so as to ensure that the CSs are the actual hand postures recognised via visual images, rather than the geometric features of the images. In fact, the CSs could be considered to be a kind of implicit motor imagery task – the process of recognising a particular hand posture, regardless of the orientation in which it is pictured (Bowering et al. 2013; Moseley 2004). The CS+ and CS- are allocations at opposite ends of the hand posture range of motion, i.e., if the CS+ is fully flexed, then the CS- is fully extended. There will also be six generalisation stimuli (GSs), being six different hand postures, chosen for their position in a continuum of postures between the CS+ and the CS-, and each shown in only one orientation, and four unrelated hand postures (distractor stimuli, DSs; Figure 2).

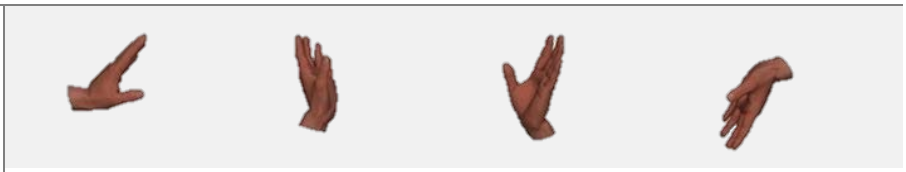
Stimulus type	**Hand posture image**
Conditioned stimuli – fully extended	
Conditioned stimuli- fully flexed	
Generalisation stimuli – range in similarity between CSs	
Distractor stimuli	

Figure 2. Depiction of the different presentations of the CSs.

Participant and experimental setup

Participants will be seated in a comfortable armchair, 600 mm from a computer monitor in a dimly-lit room. The blinded researcher will record the participant's verbal responses. The laser handpiece will be attached to a robotic arm. The robotic arm will be controlled and

moved by software (Solid Edge ST 8, CNC Mac 3 controller program, Australia) that has recorded co-ordinates, and will move the arm by 80 mm after each laser stimulation. This will be done to ensure that the laser stimulus is never delivered to the same skin location twice. The images and sequencing of the stimuli will be performed by Gentask software (Compumedics Neuroscan, El Paseo, TX). During each experimental block, the Gentask software will control the delivery of the laser stimulus when the symbol 'CS+' is displayed. A video camera will be attached to the robotic arm to provide the blinded researcher with a clear view of the hand at all times. Before the experiment, and after every block of 16 trials, an infrared thermometer will be used to check the skin temperature of the hand. This will be done as an extra safety precaution to ensure that the skin temperature has not increased significantly (more than 2°C).

Laser settings

The experiments will use a neodymium: yttrium-aluminum-perovskite (Nd:YAP) laser of 1340 nm wavelength, 4 mm spot diameter and 5 ms pulse duration. The distance between the laser handpiece and the hand will be 150—200 mm, depending on the height of the participant's hand and the clarity of the laser.

Calibration phase

Participants will be required to report the level of pain evoked by each laser stimulus using an 11-point numeric rating scale, where 0 means "no pain", and 10 means "the worst pain tolerable" (Figure 3). The stimulus rated as a 5/10 will then be administered again to different locations on the hand, so as to ensure consistency of the chosen stimulus. The stimulus will be deemed consistent if the participant rates it as '5' at two of the three locations. If participants' rating of the laser stimulus varies then the stimulus will be adjusted until a consistent rating of 5/10 is obtained. A stimulus intensity that consistently elicits a reported pain level of '5' (moderate pain) will be established and then assigned as the US (painful) and paired with the CS+ in the Acquisition phase.

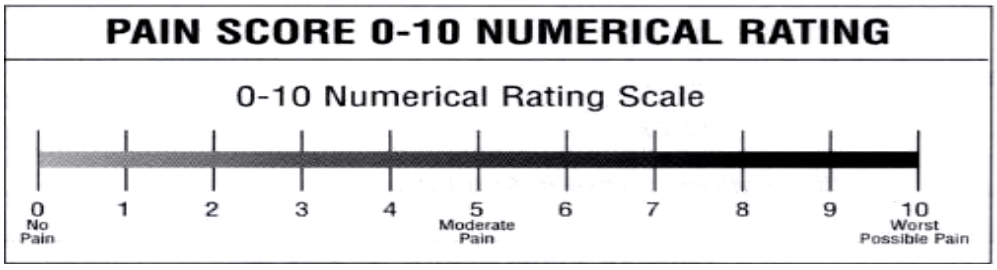


Figure 3. Numerical pain rating scale.

Group assignment

Participants will be allocated to either Group A or Group B, to allow the CS+ and CS- to be counterbalanced. For Group A, the CS+ will be a fully-flexed hand and the CS- will be a fully-extended hand. For Group B, the allocation will be reversed. The order in which the images are presented will be semi-randomised with the restriction that the same conditioned stimulus (e.g. a fully-flexed hand) cannot be displayed in the same or different orientations in consecutive trials.

Acquisition phase – three blocks of 16 trials (Attachment One)

The Acquisition phase comprises three blocks. Half of all Acquisition phase trials are question trials; the remaining half are non-question trials. Blocks 1 and 2 include both question and non-question trials. Block 3 includes question trials only. Stimuli are selected from a pool including CS+, CS- and DSs.

Trial procedure (flow of a single trial - see Figure 4)

1.) Participant sits in front of computer screen
2.) Fixation cross is displayed (duration 5 seconds) to prompt participant that the experimental block is beginning.
3.) Image displayed (CS+, CS- or DS) in the middle of the screen (duration 5 seconds).
    IF trial is a 'question trial' (see above), question displayed is: "How much do you expect the painful stimulus?". Participant rates US expectancy (expectation of the painful stimulus) using 11-point scale: 0, 'not at all' to 10, 'very much'.
    IF trial is a 'non-question trial', image is displayed alone.
    IF image is a CS+, US delivered as image disappears (delay timing).
4.) Inter-trial interval (duration 5 seconds). Next trial.

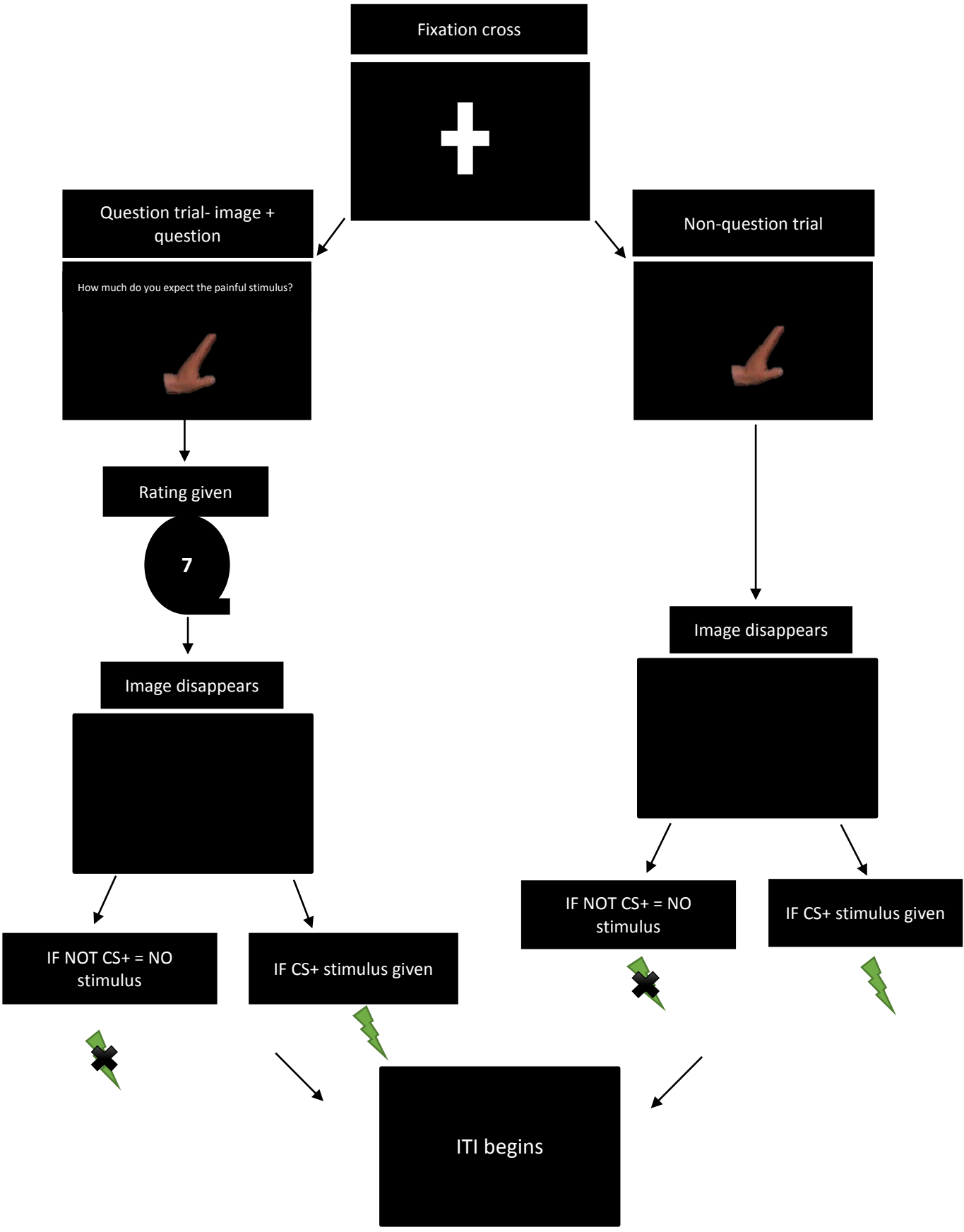


Figure 4. Trial procedure.

Fear rating

At the end of each experimental block of 16 trials, a question will be displayed in the middle of the screen: 'How fearful are you?'. Participants will be required to rate the question using a numerical 11-point rating scale: 0, 'not at all', to 10, 'very fearful'.

Generalisation phase - 1 block of 12 trials

This phase will consist of one block of 12 trials. In this experimental block, the participant will be presented with six GSs, one original CS+ and CS-, as well as two DSs.

Block 1

During the Generalisation phase, the procedure will be similar to the last block of the Acquisition phase, except in this phase, six generalisation stimuli (GSs) will be added and all but two DSs will be removed. The GSs will be of images depicting novel hand postures that vary in similarity between the CS+ and CS-. Restrictions will be placed on the sequencing of the trials, such that no more than two consecutive trials may have the same stimulus type or orientation.

Extinction phase: One block of 16 trials

The Extinction phase procedure will follow exactly the same outline as the Acquisition phase, with the exception that the CS+ and CS- will no longer be followed by the US.

Hand posture order

After completion of the experiment, subjects will be given all images (six GS, one CS+ and one CS-) and they will be asked to arrange the images in a sequence that is meaningful to them.

An awareness test will be completed to determine the level of learning that has occurred during the Acquisition phase. This will be done by presenting participants with the six GSs and the CS+ and CS-. The participants will then be asked to identify the dangerous hand posture (i.e. the image that was linked with the laser stimulus) and the safe hand posture (i.e. the image that was not linked with the laser stimulus). Participant's awareness will then

be rated depending on how close the chosen image was to the correct image (scale of 8), i.e. a participant will be classified as 'aware' if the image they pick is either the correct one (8) or the next similar one (7). The participant will be classified as 'unaware' if they score six or lower.

Questionnaires

Participants will be asked to fill out the Positive and Negative Affect Scale (PANAS), Catastrophising Assessment Tool (CAT) and State-Trait Anxiety Inventory (STAI) questionnaires. This will allow us to examine the influence of other variables that may affect the perception of pain. The PANAS was selected because negative affect has shown to increase pain perception and positive affect to decrease pain perception (Meulders, Meulders, & Vlaeyen, 2014). The CAT is used because catastrophising can lead to over-estimation of the probability of pain (Hood et al. 2012). In this study, the probability of pain will be the primary outcome; therefore, catastrophising may alter the ratings and will therefore be measured (Turner, Mancl, & Aaron, 2004). Lastly, trait anxiety can influence an individual's perception of pain and will therefore be recorded (Ploghaus et al. 2001).

Manipulation checks

Predicted pain and fear: Participants will be asked to rate the predicted pain throughout the experiment, as well as rating their fearfulness at the end of each experimental block using a numerical 11-point rating scale.

Before the experiment, participants will be informed about the importance of concentrating. After each experimental phase, participants will be asked questions regarding whether they were able to concentrate during the experiment ('Do you feel you were able to pay attention for the whole block?', and 'Did you look at each individual image?'). If the participant states that they were unable to maintain attention during that phase of the experiment, then that participant's data will be excluded from the study.

Proposed data extraction and statistical analysis

Parametric analysis

All data will be recorded in Microsoft Excel andanalysed using SPSS Version 21.0 (IBM, 2012).

1: Generalisation of predicted pain (primary analysis): A one-way repeated measures ANOVA with eight levels will be used to determine the influence of the pictures of the eight different hand postures on predicted pain ratings. We expect an interaction between images 8 (CS+) and 7 (GS 6) and predicted pain.

Exploratory analyses

2: Contingency and generalisation: a two-way mixed ANOVA with a between-subjects factor of group (aware / unaware) and a within-subjects factor of hand posture (8 levels) will be used to determine the influence of contingency on the generalisation of pain prediction. We expect steeper generalisation gradients in the aware group than in the unaware group.

3: Contingency and conditioning: Three-way ANOVA- block (2: First / final Acquisition block) x group (2: aware/unaware) x stimulus type (2: CS+/CS-). We expect a greater 3-way interaction between block and stimulus type for the aware group than for the unaware group.

4: Fear and conditioning: Two-way ANOVA of block (first/final Acquisition block) x group (aware/unaware). We expect a difference in fear ratings between the aware and unaware groups for the final Acquisition block, but not for the first Acquisition block (Field, 2009).

Normality

The data distributions were tested for normality via examination of Q-Q plots and Kolmogorov-Smirnov tests of 0.192 ($p < 0.001$ significance). All data was then transformed and normality was re-tested. The following non-parametric tests are proposed.

Non-parametric analysis

***1*:** Generalisation of predicted pain (primary): Friedman's ANOVA will be used to analyse the following three groups: CS plus, CS minus, and hand posture 6. The outcome repeated

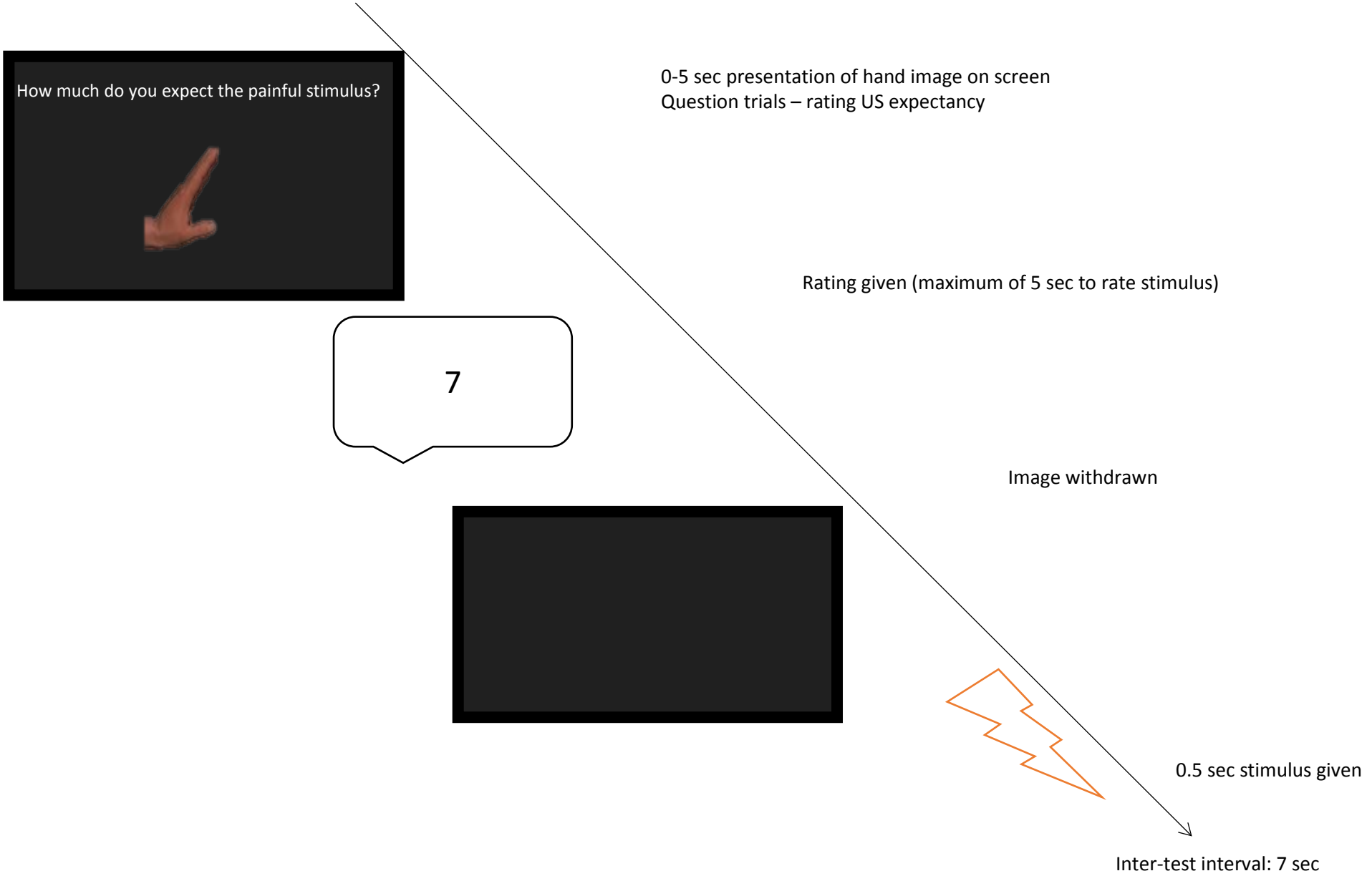
measure is 'predicted pain'. To explore the extent of generalisation from the conditioned stimulus to the novel unconditioned stimuli, a Wilcoxon signed ranked test will be used to compare the difference between the CS- and each hand posture (five hand postures in total). As this is a further exploration of our primary hypothesis, Bonferroni correction will not be used.

2: Contingency and generalisation: Kruskal-Wallis tests will be used to investigate the influence of contingency on generalisation. A comparison between aware and unaware contingency groups and their predicted pain ratings for the eight different hand postures will be investigated. We hypothesise that the aware group will demonstrate a greater awareness between the conditioned stimuli and unconditioned stimuli than the unaware group. A Mann-Whitney test will be used to examine the difference between the aware and unaware group for the hand posture conditions CS+, CS- and hand postures 7 and 6. A Jonckheere trend test will be used to analyse the trends between the means for each hand posture condition. We hypothesise that there will be a decrease in the means from CS+ to hand posture 7 to hand posture 6 through to CS-.

3: Contingency and conditioning: Kruskal-Wallis tests will be used to investigate the influence of the contingency on conditioning. A comparison between the two groups (aware and unaware) and their predicted pain ratings for the two conditioning stimuli CS+ and CS- for the first and final phases of acquisition will be conducted. We hypothesise that the aware group, in comparison to the unaware group, will have a greater change in their predicted pain ratings for both conditioning stimuli in the final phase of acquisition.

4: Contingency and Fear: Kruskal-Wallis tests will be used to investigate the influence of contingency on state fear. A comparison between two groups (aware and unaware) and their state fear ratings for the first and final phase of acquisition will be conducted. We hypothesise that the unaware group, in comparison to the aware group, will have higher rating of fear for the final phase of acquisition.

Attachment one: Question trial of CS+



Attachment two: Illness and medication temperature sensitivity

Illness – temperature sensitivity:

- Menopause
- Perimenopause
- Infection
- Common cold
- Flu
- Thyroid disorders
- Psychiatric conditions – depression, seasonal affective disorder
- Autoimmune thyroid disorders
- Raynauds phenomenon

Medication – temperature sensitivity:

- Antibiotics – examples: ciprofloxacin, tetracycline
- Antihistamines - Benadryl
- Cardiac drugs - Cordarone, Procardia
- Diuretics - Furosemide
- Diabetic drugs
- Painkillers
- Acne medication- isotretinoin, soriatan

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12.4. Appendix 4. Chapter 5 experimental protocol 2

Working title of study: Generalisation of hyperalgesia on hand postures in healthy controls.

Kerwin A Talbot[1] , Victoria J Madden[2] , Daniel S Harvie[3], Valeria Bellan[4], Sara Jones[5], G Lorimer Moseley[6]

Protocol description

This is a protocol for an experiment. The primary objective is: To determine the extent of generalisation of conditioning-induced hyperalgesia in healthy controls.

Introduction

Evidence for classical conditioning in pain-related fear and avoidance behavior in people with chronic pain is well established (Meulders et al. 2011, 2012; Meulders & Vlaeyen 2012). Studies that have used classical conditioning paradigms have demonstrated that repeated pairing of a neutral stimulus (conditioned stimulus; CS) with either an aversive or rewarding stimulus (unconditioned stimulus; US) can result in the CS eliciting responses that are associated (intuitively) with the US, such as fear and/or avoidance. The theoretical application of classical conditioning to a pain event seems logical. If researchers were to repeatedly pair a non-noxious stimulus CS and a noxious pain-related stimulus (unconditioned stimulus; US) then the CS may cause or contribute to pain as a conditioned response. The potential role of associative learning in chronic pain has recently been proposed by Moseley and Vlaeyen (2015).

Cross-over of classical conditioning theory into pain research has received less attention than research into pain-related fear and avoidance. A systematic review indicated that hyperalgesia could possibly be classically conditioned. A small number of studies have shown that a nociceptive stimulus can be more painful if it follows a pain-associated cue (Madden et al. 2016). Similarly, studies have also demonstrated that hypoalgesia can be classically conditioned. That is, a nociceptive stimulus may be less painful if it followed a cue associated with safety (Jensen et al. 2012, 2015). Pain-related fear and avoidance behaviour studies have demonstrated this modulation of the conditioned response. Moreover, they have shown that the modulation effect occurs in a graded fashion. Several studies have demonstrated that, as the similarity of new stimuli and the initial stimulus that evoked the highest pain-related fear decreases, so too does the pain-related fear, thus demonstrating a generalisation gradient (Meulders et al. 2014a, 2015; Meulders & Vlaeyen 2013a, 2013b). However, evidence of whether hyperalgesia generalises to other similar stimuli is lacking. This study will investigate (i) whether hyperalgesia can be conditioned using associative learning of painful and non-painful hand postures, and (ii) whether the effects generalise to similar hand postures.

Aim

To determine the extent of generalisation of hyperalgesia to hand posture images that differ from the hand posture image (CS+) that was originally paired with a painful US.

Objectives

1: To investigate if the pain intensity rating of a noxious stimulus (laser) increases when delivered with a CS+ hand posture image, and whether this effect generalises to similar hand posture images.

2: To investigate if the pain intensity rating of a noxious stimulus (laser) decreases when delivered with a CS- hand postures image, and whether this effect generalises to similar hand posture images.

3: To investigate whether contingency awareness modulates the generalisation of pain intensity.

Methods

Recruitment

Based on previous studies using similar methods, a sample size was calculated *a priori*. To detect an effect size of 0.4 with a power of 80% and alpha of 0.05, it was calculated that this study needed to recruit 16 participants. These participants also participated in our previous experiment ("*The generalisation of predicted likelihood of pain to similar hand postures in healthy controls*"). All potential participants were recruited via flyers, word of mouth and through the host university. Potential participants were screened for the following exclusion criteria: neurological diseases, psychiatric illnesses, current chronic pain (pain which has lasted longer than 12 weeks and present on most days (Green, 2013), recent history of chronic pain (within the last 6 months), current pregnancy, current pain (any site), recent hand injury or inability to communicate effectively using English. As a neodymium: yttrium-aluminum-perovskite (Nd: YAP) laser was used, additional exclusion criteria were deemed necessary to ensure participant safety. These were: illness (minor or major), medications that may alter skin sensitivity, altered skin sensation (e.g. peripheral neuropathy of the hand, significant scars or burns, Raynaud's disease) and skin conditions on the upper limb and hand (e.g. eczema). Volunteers who had recently participated in conditioning experiments were excluded, except if it was longer than six months ago, and the nature of

the conditioning experiment was very different to the current experiment (i.e. different stimulus and different delivery of stimulus).

Ethics and consent

This study was approved by the University of South Australia Human Research Ethics Committee (0000033772). Eligible participants were included in this study after they gave informed consent. Participants were given a verbal explanation of how the experiment would run and any questions were answered. At this point, it was reiterated to participants that they may withdraw from the study at any time without judgement.

Materials and measures

This experiment had three different phases, an Acquisition phase (consisting of 3 blocks of 16 trials), a Generalisation phase (consisting of 1 block of 12 trials) and an Extinction phase (consisting of 1 block of 16 trials; Table 1).

Table 1. Experimental outline of each phase's stimuli and responses.

	Stimulus type							**Rating**	
Phase	**CS+/ US_H**	**CS+ / US_M**	**CS+ alone**	**CS- alone**	**CS-/US_M**	**GS/ US_M**	**DS alone**	**US-expectancy (pain)**	**Pain intensity**
ACQ 1	4 T			4 T			8 T		
ACQ 2	4 T			4 T			8 T		
ACQ 3	4 T			4 T			8 T		
GEN		2 T			2 T	6 T			
EXT			4 T	4 T			8 T		

Note: ACQ: Acquisition; GEN: Generalisation; EXT: Extinction; US_H: high stimulus; US_M: moderate stimulus, T: trial, shaded area: if trial is to occur during that phase.

Conditioning stimuli

Different hand posture images were used as conditioned stimuli (CS), generalisation stimuli (GS) and distractor stimuli (DS; Figure 1). The CS+ and CS- were assigned to two specific hand postures: a fully flexed hand and a fully extended hand. The GS were six images of hand postures that ranged between the CS+ and the CS-. The distractor stimuli were four unrelated hand postures used to make the task more difficult. Participants viewed four

different orientations of the CS+ and the CS-, in order to ensure that the CS's are the actual hand postures recognised via visual images, rather than the geometric features of the images. The GS and DS were displayed in one orientation.

Unconditioned stimulus

A neodymium: yttrium-aluminum-perovskite (Nd:YAP) laser (1340 nm wavelength) was used as the unconditioned stimulus. A 4 mm spot diameter and 5 ms pulse duration was used. Spot size was calibrated before testing each participant. The distance between the laser and the hand was 150—200 mm, depending on the height of the participant's hand (this was factored in during calibration) and the clarity of the laser.

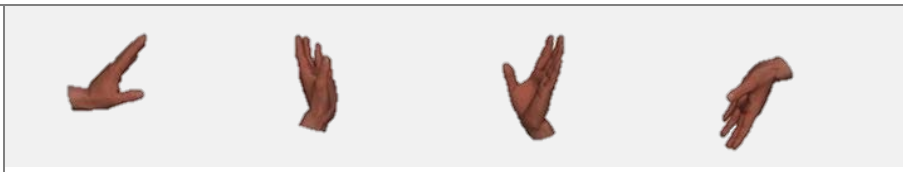
Stimulus type	Hand posture image
Conditioned stimuli – fully extended	
Conditioned stimuli- fully flexed	
Generalisation stimuli – range in similarity between CSs	
Distractor stimuli	

Figure 1. Hand posture images used for conditioning stimuli.

Participant and experimental setup

Participants were seated in a comfortable armchair, at a distance of approximately 600 mm from a screen in a dimly-lit room. The participant was required to wear protective goggles as a safety measure associated with the use of the laser. Two researchers were involved in the conduct of the experiment. The first researcher (who was blinded to the experimental hypotheses) recorded the participant's verbal responses. The participant's right hand was shielded from view and placed on a high density flat foam cushion. A 70 mm square was mapped out on the dorsum of the right hand using skin markers. The laser handpiece was attached to a robotic arm that was controlled and moved by software (Solid Edge ST 8, CNC Mac 3 controller program, Australia). The software had pre-programmed co-ordinates which moved the arm by 8 mm after each laser stimulation. Moving the laser after each stimulation ensured that the same skin location was not repeatedly stimulated. Each experimental block was pre-programmed using Gentask software (Compumedics Neuroscan, El Paso, TX). The Gentask software controlled the delivery of the images (displayed on the participant's screen) and the laser stimulation (via TTL), which was only delivered when the CS+ image was displayed. Two video cameras were used; one was attached to the robotic

arm to provide the researcher controlling it with a good view of the hand at all times. The second camera was attached to the participant's screen. This allowed the second researcher to observe the participant. As an additional safety precaution, an infrared thermometer was used to check the skin temperature of the participant's hands. Detection of a significant difference in temperature (greater than 2°C) between the non-stimulated and stimulated hand was used as an indicator that a longer interval should be used between blocks. If the difference continued to be detected, the experiment was ceased.

Calibration phase

Participants were given the laser stimulus in pulses with gradually increasing intensity, starting from the lowest setting. Using an 11-point numerical rating scale where 0 means "no pain", and 10 means "the worst pain tolerable", participants rated each stimulus, until a pain rating of 5/10 was reached. To ensure consistency of the rating, the same stimulus was administered to three different locations. The stimulus was deemed consistent if the participant rated it as 5/10 at at least two of the three locations. If the ratings were inconsistent, then the stimulus intensity was adjusted accordingly and the process repeated until a consistent pain level of 5/10 was elicited. Once the 5/10 rating was established, it was assigned as the US (painful) and paired with the CS+.

Rating scale

Numerical rating scales were used during the experiment and calibration. Participants used an 11-point numerical rating scale to rate pain intensity. In this the anchors used were 0, meaning "no pain", and 10 meaning "the worst pain tolerable" in response to the question "How painful is the stimulus?" To rate the predicted likelihood of pain, an 11-point numerical rating scale was used. The anchors were 0, "not at all", and 10, "very much", in response to the question "How much do you expect the painful stimulus?" At the end of each experimental block, the participant rated their fear level. In this instance, the anchors used were 0, "not at all", and 10, "very fearful", and the question was "How fearful are you?"

Counterbalancing and randomisation

The CS+ and CS- were counterbalanced; therefore, participants were allocated to either Group A, where the CS+ was a fully flexed hand and the CS- was a fully extended hand; or to Group B, where the allocation was reversed. The orders of the images were semi-randomised, with the restriction that the same CS was not displayed in the same or different orientations in consecutive trials. Semi-randomisation was done using Microsoft Excel. The hand posture images were assigned a random number. The Excel function was then used to randomise blocks of eight hand posture images at a time. Nine different semi-randomised Acquisition blocks were created, that is, for each Acquisition block, three different semi-randomised hand posture images blocks were available (Figure 2). A similar process was undertaken for the generalisation and Extinction phases, although there were only two different semi-randomised blocks available.

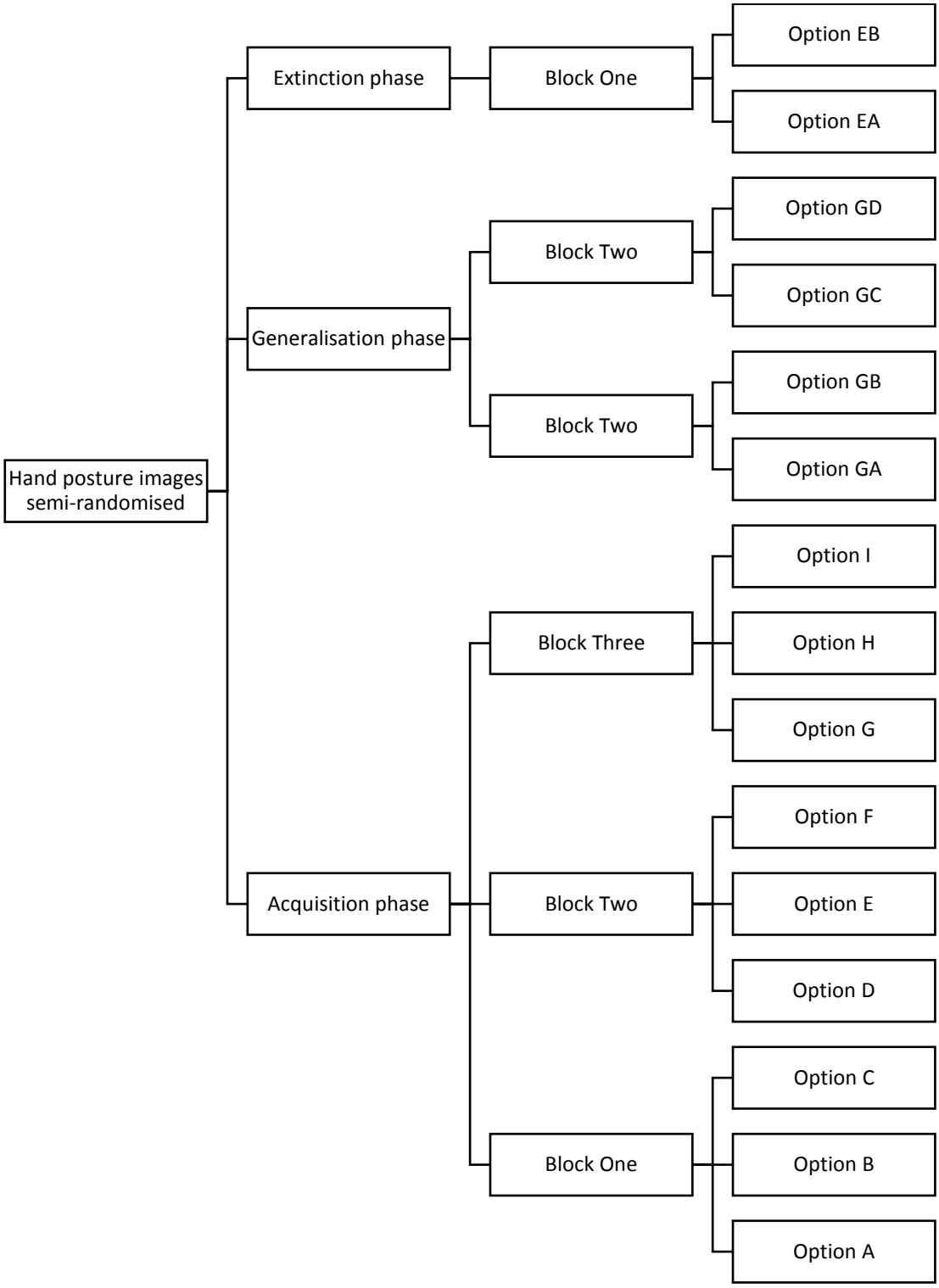


Figure 2. Hand posture image semi-randomisation process.

Participants were randomly assigned one of the options for each block. For example, Participant One received option A for Acquisition block one, option F for Acquisition block two and option H for Acquisition block three, option GB for Generalisation block one, option GC for Generalisation block two and option EB for the Extinction phase. Randomisation for each participant was done before any recruitment began.

Acquisition phase

The Acquisition phase comprised three blocks of 16 trials each. Half of all the Acquisition phase trials were question trials and the remaining half were non-question trials. Question and non-question trials were presented in blocks one and two, but only question trials were presented in block three. Stimuli were selected from a pool including CS+, CS- and DSs.

Trial procedure (Single trial flow - see Figure 3)

1. Participant sat in front of computer screen.
2. Fixation cross was displayed (duration 5 seconds) to prompt participant that the experimental block was beginning.
3. Image displayed (CS+, CS- or DS) in the middle of the screen (duration 5 seconds).
   IF trial was 'question trial' (see above), question displayed: "How much do you expect the painful stimulus?" Participant rated US expectancy (expectation of the painful stimulus) using 11-point scale: 0, 'not at all' to 10, 'very much'.

IF trial was 'non-question trial', image was displayed alone.

IF image was CS+, US delivered as image disappeared (delay timing).

4. Inter-trial interval (duration 5 seconds). Next trial.

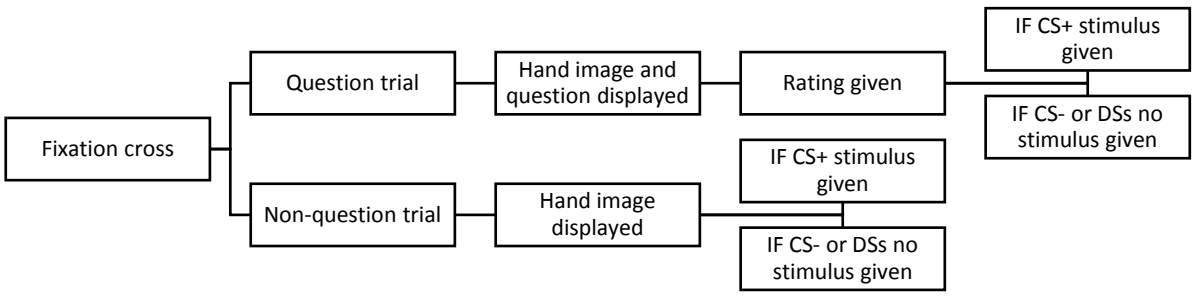


Figure 3. Question and non-question single trial flow.

Generalisation phase - one block of 12 trials

This phase consisted of one block of 12 trials. In this experimental block, the participants were presented with six GSs, one original CS+ and CS-, as well as two DSs. During the Generalisation phase, the procedure was similar to the last block of the Acquisition phase, with the exception that in this phase, six GSs were added and all but two DSs were removed. The GSs were of images depicting novel hand postures that varied in similarity between the CS+ and CS-. All visual stimuli (CS+, CS- and GSs) were accompanied with a moderate stimulus that had been previously rated during calibration as 3/10.

Trial procedure (single trial flow - see Figure 4)

1. Participant sat in front of computer screen.
2. Fixation cross was displayed (duration 5 seconds) to prompt participant that the experimental block was beginning.
3. Image displayed (CS+, CS- or DS) in the middle of the screen (duration 5 seconds).
4. Image disappeared.
5. Moderate stimulus given (delayed 0.33 ms).
6. Question displayed “How painful is the stimulus?”, 0 means “no pain” and 10 means “the worst pain tolerable”.
7. Participants rated the stimulus.
8. Inter-trial interval (duration 5 seconds). Next trial.

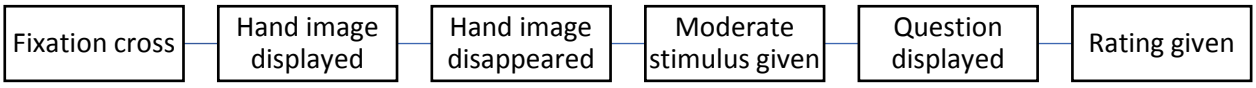


Figure 4. Generalisation single trial flow.

Extinction phase -One block of 16 trials

The Extinction phase procedure followed the same outline as the Acquisition phase, with the exception that the CS+, CS- and DSs was no longer followed by the US.

Hand posture order

After completion of the experiment, subjects were given all images (6 GSs, 1 CS+ and 1 CS-), and asked to arrange the images in a sequence that was meaningful to them.

Contingency awareness

Contingency awareness is defined as the participant's "…knowledge of the reinforcement contingencies in the experiment". That is, whether the participant has learned that the CS+ is paired with the US (high painful stimulus) and the CS- is unpaired. Contingency awareness was assessed in two ways. First, a data analysis was performed, where Acquisition block 3 predicted likelihood of pain rating for the CS+ will be subtracted from the CS- rating. A result of greater than 8 (CS+ - CS- > 8) indicates the participant is considered contingency aware of the CS+ and CS-. A result of less than 8 (CS+ - CS- < 8) indicates the participant is considered contingency unaware of the CS+ and CS-.

Second, at the end of the procedure, a retrospective questionnaire was undertaken, where participants were given pictures of each image (CS+, CS- and GSs) and asked 'How much did you associate this hand with the high painful stimulus?' with 0 indicating 'not at all' and 100 indicating 'very much'. Participants were required to give a rating of 80—100 for the CS+ and a rating of 0—20 for the CS- to be classified as 'contingency aware'. Any participant whose ratings were outside of these ranges were classified as 'contingency unaware'. However, the retrospective question does not provide a measure of contingency awareness during the experiment. The question may therefore only be an indicator of extinction.

Questionnaires

Participants completed the Positive and Negative Affect Scale (PANAS), Catastrophising Assessment Tool (CAT) and State-trait Anxiety Inventory (STAI) questionnaires. These questionnaires allowed examination of the influence of other variables that may affect pain. The PANAS was selected because negative affect has shown to increase pain, and positive affect to decrease pain (Meulders et al. 2014b). The CAT was used because catastrophising can lead to over-estimation of the probability of pain occurring (Hood et al. 2012). In this study, the pain intensity ratings were the primary outcome (Turner et al. 2004). Lastly, trait anxiety can influence pain and therefore was recorded (Ploghaus et al. 2001).

Manipulation checks

Participants were asked to rate the predicted likelihood of pain throughout the experiment, as well as rating their fearfulness at the end of each experimental block, using a numerical 11-point rating scale.

Before the experiment, participants were informed about the importance of concentrating. After each experimental phase, participants were asked questions ('Do you feel you were able to pay attention for the whole block?' & 'Did you look at each individual image?') to assist in identifying whether they were able to concentrate during the experiment. If the participant stated that they were unable to maintain attention during that phase of the experiment, then that participant's data were excluded from the study.

Lodging protocol

The experimental protocol and analysis plan will be lodged and locked on the Open Science Framework prior to commencement of data analysis.

Data extraction and statistical analysis

All data was recorded in Microsoft Excel andanalysed using SPSS Version 21 (IBM, 2012).

Normality

Distribution will be analysed using a Shapiro-Wilk test. There is a high expectation that the data collected will have high proportions of 0 ratings, which could skew the data distribution beyond normality. If the distribution of the data is skewed and non-normal, then the skewness statistic will be assessed. If the statistic is between 1 and -1, then a parametric test will be used. If the skewness static is outside that range, then the data will be transformed and the new distribution assessed using the same tests. However, if, after transforming the data and re-testing it, the data are still found to be non-normally distributed and outside the skewness parameters, then non-parametric tests equivalent to the parametric tests detailed below will be used.

Parametric analysis

When appropriate, Mauchly's test will be used to assess sphericity, if the assumption of sphericity is violated, then the appropriate correction will be reported, such as the Greenhouse-Geisser statistic. Bonferroni correction will be used to minimise the risk of type-one error.

The criterion for conditioned amplification will be a CS+ vs. CS- difference. That is, to determine whether generalisation has occurred, the pain rating for the first CS+ must be greater than that of the CS- . If the CS+ is not greater than the CS-, then it will be deemed that generalisation of amplified pain has not been demonstrated. In this case, no further analysis will occur. If this is observed (CS+ more painful than CS-) then at least 1 GS (GS 6) must be greater than the CS-. Following this, if both results are observed (CS+ is greater than the CS- and GS6 is greater than CS-) then the other pain intensity ratings for the GSs will be paired (GS1&2, 3&4, 5&6). These will be contrasted against the CS- pain intensity ratings. Effects in both the overall results and *post hoc* contrasts will need to be observed to demonstrate generalisation of amplified pain.

Exploratory analysis

A two-way mixed ANOVA with a between-subjects factor of group (aware/unaware) and within- subjects factor of hand posture (8 levels) will be used to determine the influence of contingency on generalisation of amplified pain.

Planned secondary analyses of relationships between questionnaire results and findings will be conducted. The raw scores for each questionnaire (PANAS, STAI and CAT) will be used. Participants will be separated into a high group and a low group for each questionnaire. Participants with the five highest scores will make up the high group (i.e. high positive affect group, high negative affect group, high CAT group) and the participants with the five lowest scores will make up the low group (i.e. low positive affect group, low negative affect group, low CAT group). Re-analysis of the two subgroups' (high positive affect group vs. low positive affect group) generalisation of pain intensity and hyperalgesia will be completed and compared. The results from this analysis will be used to inform future hypothesis generation.

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12.5. Appendix 5. Chapter 7 experimental protocol

Working title of study: Generalisation of amplification of pain in healthy people

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Protocol description

This is a protocol for an experiment. The primary objective is as follows: To determine the extent of generalisation of conditioning-induced hyperalgesia to faces.

Introduction

Previous fear-related studies have demonstrated that pain-related fear that is initially associated with one stimulus (which may be visual or a movement) can generalise to other novel but similar stimuli. That is, if the initial stimulus (a joystick movement to the left), known as a conditioned stimulus (CS+), has been paired with a painful heat stimulus, known as an unconditioned stimulus (US-painful), it may come to evoke a pain-related fear response, known as a conditioned response (CR; Meulders & Vlaeyen 2012). This CR has been shown to also be evoked by similar stimuli, a process known as generalisation stimuli (GS), and the CR decreases as the similarity of the GS to the initial CS+ decreases. Similar stimuli are considered to be stimuli that resemble the initial one, in the case of this study, it was joystick movements in a similar direction (Meulders & Vlaeyen 2012). Indeed, through classical conditioning studies, this generalisation has been demonstrated in pain-related fear ratings (Meulders et al. 2015) and avoidance behaviours (Meulders et al. 2011). However, it is yet to be shown in a situation in which one can choose to avoid the painful stimuli altogether. This situation has clear ecological validity. Therefore, this study will investigate the extent of generalisation of a learned behavioural response (pushing a button) that is guaranteed to prevent the experience of pain.

Several studies have demonstrated hyperalgesia and hypoalgesia when participants view faces that have previously been associated with stimuli of various intensities. Jensen et al. (2015) used a classical conditioning paradigm, in that they paired a high intensity stimulus with one face (CS+) and a low intensity stimulus with another different face (CS-). During the testing phase, however, a moderate intensity heat stimulus was given for both the CS+ face and the CS- face. When participants viewed the CS+ face, they rated the moderate-intensity heat stimulus as more painful (hyperalgesia); whereas when they viewed the CS- face, they rated the moderate-intensity heat stimulus as less painful (hypoalgesia). However, no studies have investigated the impact that face stimuli may have on the generalisation of hyperalgesia and hypoalgesia to a test stimulus paired with face stimuli that are novel but similar to the initial two conditioning face stimuli (i.e. conditioned stimulus plus, CS+, previously associated with a highly painful stimulus; and conditioned stimulus minus, CS-, previously associated with a slightly painful stimulus). Therefore, this study will also investigate the extent of generalisation of amplified pain to a test stimulus paired with face stimuli that are novel but similar to the initial two stimuli (faces).

Aims

1. To determine the extent of generalisation of US expectancy ratings in healthy people.
2. To determine the extent of generalisation of amplified pain (hyperalgesia, CR 2) using different face stimuli in healthy people.

Secondary aim

1. To determine the extent of generalisation of the learnt behavioural response (button pushes, CR) in healthy people.

Objectives

Objective one

To investigate whether US expectancy is rated higher for the CS+ than for the other face stimuli, and whether the US expectancy ratings will decrease as the similarity of the face stimuli to the CS+ decrease.

Objective two

To investigate whether contingency awareness influence US expectancy ratings for different face stimuli. This will have two components:

a. To investigate the amplification of pain (CR 2) to images of faces (GSs) that are of graded similarity to the originally painful face image (CS+).

b. To investigate whether the amplification of pain (CR 2) is associated with the CS+ and CS- neutral faces.

Secondary objectives

a. To investigate whether the behavioural response (CR 1) is learned by association with the CS+ (neutral face) but not with the CS-.

b. To investigate whether the learnt behavioural response (CR 1) associated with the CS+ and CS- is modulated by images of faces of varying similarity (generalisation stimuli, GS) to the originally pain-associated neutral face (CS+).

c. To investigate whether contingency awareness is associated with the difference in the behavioural response (CR 1) associated with the CS+, CS- and GSs.

d. To investigate whether the learnt behavioural response (CR 1) is extinguished when no longer associated with the painful US.

Methods

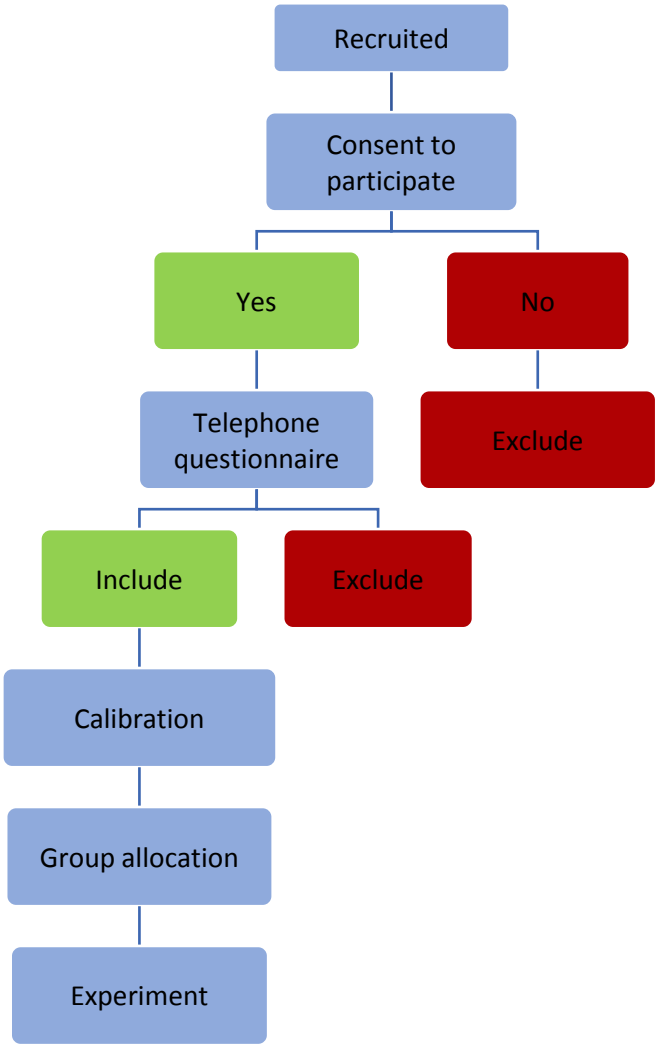


Figure 1. Overview of participant recruitment process.

Participants

This study will recruit a convenience sample of 24 participants through flyers and word of mouth at the University of South Australia. The targeted sample size is based on an *a priori* calculation (ES: r = 0.4; power of 80% and α = 0.05) using G*power (Faul, Erdfelder, Buchner & Lang, Institute of Psychology, University of Duesseldorf, Version 2, Germany). The specific outline for the sample size calculation, which was based on a previous study by Meulders et al. (2015), is shown in Attachment 1. That study used similar methodology and analyses as this study proposes to use. All volunteers will be given an honorarium of AUD $20 per hour for their time. All potential participants will be screened for any neurological disease, pain, pregnancy, injury (specifically hand injuries), major or minor illnesses (such as neurological, psychiatric, autoimmune, cold and flu, gastrointestinal), medication (which would alter skin sensitivity, have an analgesic effect or have effects on vision), heart conditions (including

pacemakers, due to the administration of electrocutaneous stimuli), altered skin sensitivity, skin conditions (particularly located on the hand—site of stimulation), and ability to communicate effectively using English. These exclusion criteria have been selected as the hypotheses are still being established and, therefore, investigation in healthy participants is required before the hypotheses can be tested in clinical populations. As a classical conditioning paradigm is to be used, any participant who has participated in a classical conditioning experiment in our lab within the last six months will be excluded. The experimental protocol was approved by the University of South Australia, Human Research Ethics Committee (HREC), protocol number: 0000033772. All participants will be required to sign a consent form and will be informed that they can withdraw from the experiment at any time.

Stimulus material and measures

Software: The experiment will be programmed in Affect 4.0. (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010).

Conditioned stimuli: The conditioning stimuli to be used will be taken from the Karolinska Directed Emotional Faces (KDEF) set (www.emotionlab.se/ resources/kdef). This is a set of 4900 images developed for the use in perception, attention, emotion, memory and masking experiments. The set consists of pictures of 70 individuals (35 female, 35 male; mean age: 25 years; age range: 20—30 years) with 7 different facial expressions per individual. The images to be used in this experiment will be neutral expressions, that is, they will have none to minimal emotional valence. They have been used in multiple other studies. A rigorous method was followed when taking the KDEF images to minimise any other distracting factors (consistent clothing, lighting, distance and position relative to the camera). Furthermore, a study conducted by Goeleven et al. (2008) evaluated the KDEF for its emotional content and demonstrated that the database contains a valid set of affective facial pictures. The generalisation stimuli (GSs) will be created using the Face Film application (by author KAT, using the KDEF database; version 3.3.20; Yan 2016)) run on a Macbook computer. This will allow elements of the conditioned stimuli to be mixed to create images that vary in similarity between the CS+ and CS- (see Figure 2). In the Generalisation phase (Phase 2) a control face will be added. This will be a neutral face as well, and will only be shown to participants in Phase 2.

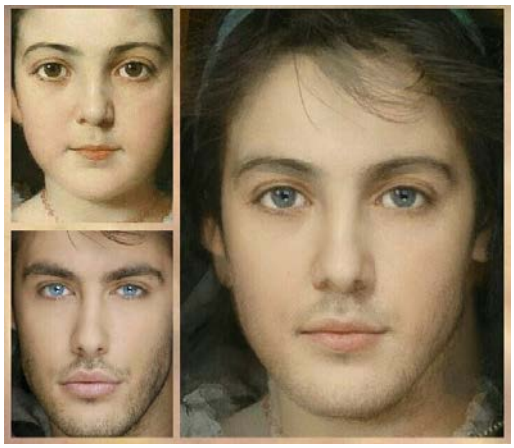


Figure 2. Example of face blending using the Face Film application (version 3.3.20; Yan 2016).

The CS+ image and CS- image will be counterbalanced (half the participants will receive one face (face A) as the CS+ and another face (face B) as the CS-, the other half will receive the reverse- CS+ (face B) and CS- (face A)). Neutral faces will also be used as distractor stimuli (DSs) which will be included in the Acquisition phases to make the task more difficult.

Face A Face B

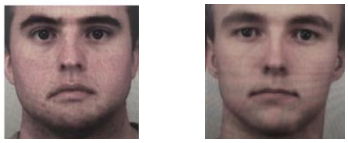


Figure 3. Images of two different faces used as conditioning stimuli.

Unconditioned electrocutaneous stimulus

Multiple stimuli are required to be administered to the dorsum of the hand as an US. Electrocutaneous stimulation was considered to be the most practical, and has a minimal safety risk. The unconditioned stimulus (US) will be a highly painful (this will be determined during calibration) electrocutaneous stimulus (high-pain US). The high-pain US will be delivered to the ventral surface of the forearm with an intracutaneous electrode, and will be paired with the CS+ image. The intracutaneous electrode will resemble that of Inui et al. (2002). The electrode has a concentric anode with a pin-type cathode in the centre. This pin-type intracutaneous electrode was chosen because low-intensity electrical stimuli produced using this arrangement are thought to produce selective activation of free nerve endings, because the current passes through the

superficial layers of the skin (Inui & Kakigi 2012). However, higher intensity stimuli may also activate non-nociceptive neurons (Mouraux et al. 2010). A non-painful, low intensity (determined in calibration), electrocutaneous stimulus (low-pain US) will be administered to the ventral surface of the forearm and will be paired with the CS-. The electrodes will be placed 40 mm apart (Figure 3). Similarly, as the face images will be counterbalanced, so too will the locations of the electrodes (half will receive the high intensity stimulus at the top location (location A) and the low intensity stimulus at the bottom location (location B) whilst the other half will receive the reverse. Similarly, the moderate intensity stimulus (moderate-pain US) will be counterbalanced; half the participants will receive the stimulus in location A and the other half in location B.

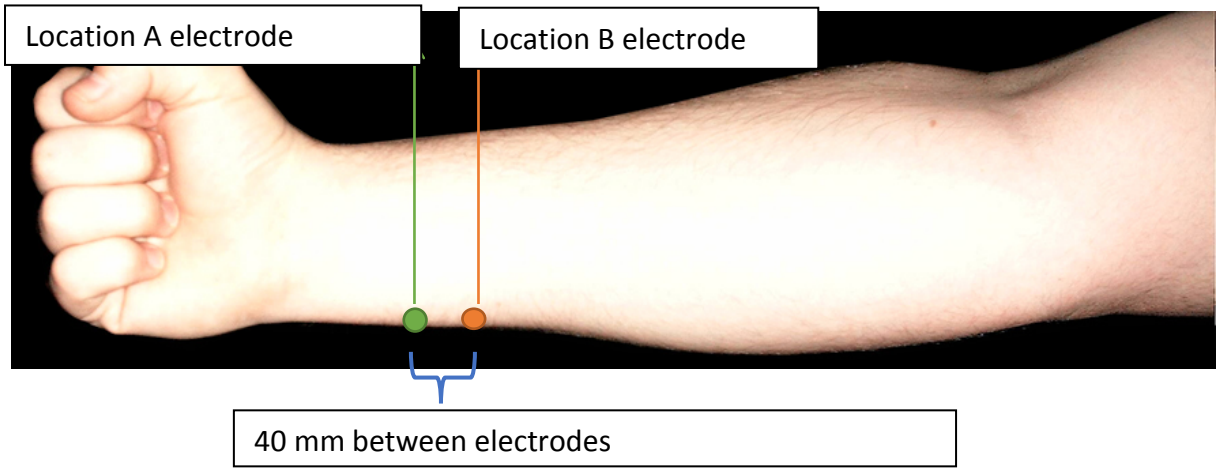


Figure 3: Electrode placement on forearm.

Electrical current will be passed across the electrodes using Digitimer devices (DS7AH, SDR Scientific, Power: 12 Va, Freq: 47-63 Hz, Hydeway Welwyn, Garden City, UK) which will be controlled by the Affect 4.0 software (Spruyt et al. 2009). During the Generalisation phase block 2 (amplification of pain), a moderately painful stimulus (determined during calibration) will be administered to the hand.

Rating scales:

Throughout the experiment, participants will rate the extent to which they predict the occurrence of pain using a 100 mm (approximately) US expectancy rating scale. The question asked will be, 'How much do you expect the painful stimulus to occur now?, and anchors used for the predicted likelihood of pain rating will be, 0, meaning 'not at all', and 100, meaning 'very much'. Participants will rate pain intensity on the Fifty Either Side of Threshold Numerical Rating Scale (FESTNRS;

Madden et al. Under review). The question and anchors used for pain intensity ratings will be, ‘Please rate your experience on the appropriate side of the scale.’, with -50 meaning ‘no sensation’, +50 meaning ‘the most intense pain you can imagine’, and 0 meaning ‘the exact point at which what you feel transitions to pain’. Two scripts have been devised (Attachment 2) and will be used to explain the use the scales to participants.

Behavioural outcome

During some experimental phases, participants will be informed that they can push a button (conditioned response 1, CR1). This button, when pushed, will prevent the delivery of the high intensity stimulus only (the stimulus they rate as highly painful). That is, the participant must recognise the face (CS+) that is (delay) paired with the high intensity stimulus. If they push the button after the CS+ has been shown, then the high intensity stimulus will be prevented, but if they push the button when the CS- or DS is shown, then the participant will still receive the low intensity stimulus. Affect will record whether the button was pushed (yes or no), the time taken to push it (response time in seconds), and whether it was constructive or unconstructive (i.e. correct = CS+; incorrect = CS- or DS).

Procedure

A within-subject experimental design will be combined with a classical (trace) conditioning paradigm to investigate the research objectives. The study will comprise a single session which will consist of several phases: Preparation, Calibration, Acquisition blocks 1, 2 & 3, Generalisation block 1, Re-acquisition, Generalisation block 2, Extinction block 1 and Extinction block 2 (Table 1). Each phase has a certain number of trials (outlined in the table as 'number' T). There are several different combinations of face stimuli (CSs, DSs and GSs) and electrocutaneous stimuli (low, moderate and high). The label given will describe the type of stimulus intensity and type of US (i.e. a CS paired with a high US will be labelled 'CS+/US_H' or a GS paired with a moderate US will be labelled 'GS/US_M'). There are three types of conditioned responses: an expectancy rating (predicted likelihood of pain); a pain intensity rating, and a behavioural response (button push). If, during a block, a particular CS or DS or GS/US $_L$ or $_M$ or $_H$ or $_{alone}$ occurs, and a particular conditioned response is recorded, then the corresponding table segment is shaded in. That is, in Acquisition phase 1 (AQ1), four trials will have the CS+ face paired with a high US, four trials will have the CS- face paired with a low US, and eight trials will have DS faces paired with a low US. The expectancy rating will also be recorded (Table 1).

Table 1. Outline of experimental procedure.

	Stimulus type													**Conditioned response**		
Phase	**CS+ alone**	**CS+/US$_H$**	**CS+/US$_M$**	**CS- alone**	**CS-/US$_M$**	**CS-/US$_L$**	**GS alone**	**GS/US$_M$**	**GS/US$_L$**	**DS alone**	**DS/US$_L$**	**DS/US$_H$**	**Control/US$_M$**	**Button option**	**Expectancy**	**P report**
AQ 1		4 T				4 T					4 T				12 R	
AQ 2		4 T				4 T					4 T			4 CBP 8 ABP	12 R	
AQ 3		4 T				4 T					4 T			4 CBP 8ABP	12 R	
GEN 1		2 T				2 T			6 T (1xGS)					2 CBP 4 ABP	10 R	
RE-AQ		4 T				4T					4 T					12 R
GEN 2			2 T		2 T			6 T (1xGS)					1 T			10 R
EXT 1	4 T			4 T						4 T				12 ABP	12 R	
EXT 2	4 T			4 T						4 T					12 R	

Note: AQ: Acquisition, GEN: Generalisation, RE-AQ: Re-acquisition, EXT: Extinction, T: trials, R: rating, CBP: correct button pushes, ABP: available button pushes.

Preparation

All participants will be informed (both verbally and in writing) that they will receive a range of painful and non-painful stimuli during the experiment. The forearm will be abraded and cleaned and the electrodes will be attached ≈40 mm apart using Micropore hypoallergenic medical tape at the two different locations. Participants will be seated facing a screen approximately 60 cm away, and a partition will be used to prevent the participant from viewing their hand or the stimulatory devices. A button will be placed near the participant's dominant hand.

Calibration

The calibration procedure will be carried out by administering a very low-intensity electrocutaneous stimulus to the participant's hand and then incrementally increasing the

stimulus intensity. After each stimulus delivery, the participant will be asked to rate it using the FESTNRS, with -50 meaning 'no sensation' and +50 meaning 'the most intense pain you can imagine' and 0 meaning 'the exact point at which what you feel transitions to pain'. A reported pain intensity rating of at least +30/+50 ('*it is painful and requires effort to tolerate'*) will be used as the high-pain US and coupled with the CS+. An intensity corresponding to a reported -10 to -20/-50 rating ('*I feel something but it's not painful*') will be used for the low-pain US and coupled with the CS-. An intensity corresponding to a reported +10 to +20/+50 ('*it is only moderately painful*') will be used for the moderate-pain US, which will be coupled with the CS+, CS- and GSs during the amplification testing phase.

Acquisition

The Acquisition phase will comprise three blocks of 16 trials, which was chosen because previous classical conditioning studies investigating similar outcomes have demonstrated conditioning using this block-trial design. The CS+ and CS- will be displayed four times each. For the first block, the only outcome will be the rating of predicted likelihood of pain (US- expectancy). For the second and third blocks, the outcomes will be (1) predicted likelihood of pain rating and (2) button-pushing behaviour (response latency; whether the button was pushed (yes) vs not pressed (no); and whether the press was constructive (CS+ trial) or unconstructive (CS- or DS trial).

Acquisition phase: Trial procedure for Block 1 (flow of a single trial - see Figure 3)

1.) Participant sits in front of computer screen

2.) Fixation cross is displayed (duration 5 seconds) to prompt participant that the experimental block is beginning.

3.) Image displayed (CS+, CS-, DS) in the middle of the screen (duration 5 seconds).

IF trial is a 'question trial', the question displayed is: 'How much do you expect the painful stimulus to occur now?' It will be emphasised to the participants that they are rating the expectation of the painful stimulus and not the low intensity stimulus. Participants rate US expectancy (expectation of the painful stimulus) using a 100-point scale: 0, 'not at all' to 100, 'very much'.

IF trial has no question attached, i.e. a non-question trial, image is displayed alone.

IF image is CS+, high-pain US delivered as image disappears (delay timing).

IF image is CS-, low-pain US delivered as image disappears (delay timing)

5.) Inter-trial interval (duration 5 seconds). Next trial.

Acquisition phase: Trial procedure for blocks 2 and 3 (flow of a single trial - see figure 3)

1. Participant given information regarding the option to push a button. Participant will be told that if they expect the painful stimulus that they can push this button which will interrupt the painful stimulus and they won't receive it. However, the participant will be told that there is only a set number of button pushes (they will not be told the exact number) and once they are all used up, then they will no longer be able to prevent the painful stimulus. It will also be emphasised to the participant that the button push will prevent only the high-pain US and will not prevent the low intensity stimulus.
2. Participant sits in front of computer screen.
3. Fixation cross is displayed (duration 5 seconds) to prompt participant that the experimental block is beginning.
4. Image displayed (CS+, CS-, DS) in the middle of the screen (duration 5 seconds).
5. Question displayed: "How much do you expect the painful stimulus to occur now?" Participant rates US expectancy (expectation of the painful stimulus) using 100-point scale: 0, 'not at all' to 100, 'very much'.

IF button is pushed

- IF correct (i.e. CS+), then no painful stimulus is given.
- IF incorrect (i.e. CS- OR DS), then low stimulus is given.

IF button is NOT pushed

- IF CS+ image, then high-pain US is given.
- IF CS- OR DS image, then low-pain US is given.

6. Inter-trial interval (duration 5 seconds). Next trial.

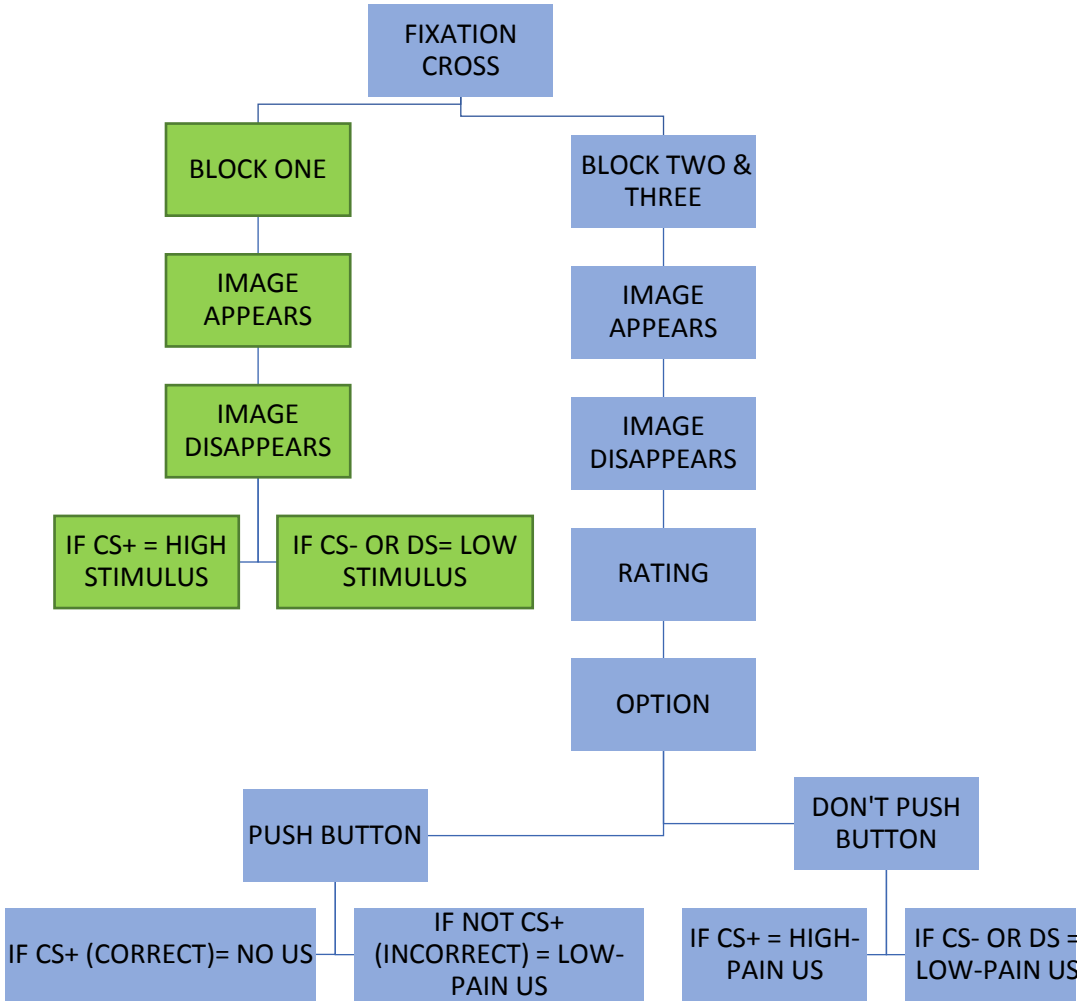


Figure 4. Acquisition phase experimental trial procedure.

Generalisation block 1 (behavioural response, CR1)

The Generalisation phase will be similar to the third block of the Acquisition phase, except that, in addition to the CS+ face image and CS- face images, 3 GS images will be included in this phase. Participants will still be allowed to push the button and will be required to rate the US expectancy using the 100 mm predicted likelihood of pain scale.

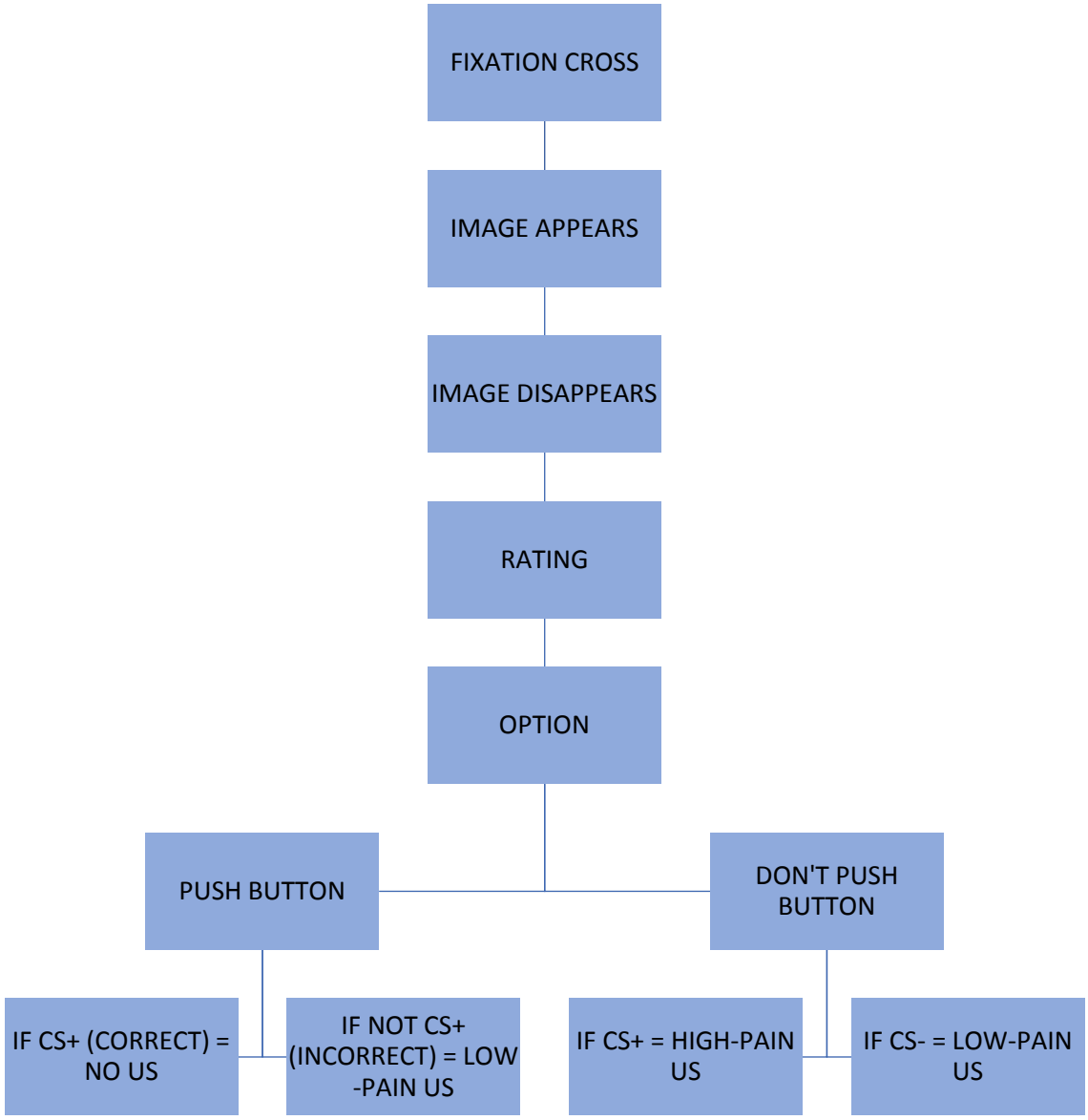


Figure 5. Generalisation of protective behavioural response experimental trial procedure.

Re-acquisition

The Re-acquisition phase will be similar to the first block of the Acquisition phase. However, participants will be asked to rate the stimulus ("Please rate your experience on the appropriate side of the scale") using FESTNRS and there will be no button push.

Trial procedure: Re-acquisition phase (flow of a single trial)

1.) Participant sits in front of computer screen.

2.) Fixation cross is displayed (duration 5 seconds) to prompt participant that the experimental block is beginning.

3.) Image displayed (CS+, CS-) in the middle of the screen (duration 5 seconds).

- IF CS+, high-pain US given
- IF CS-, low-pain US given

4.) Participant rates stimulus – "Please rate your experience on the appropriate side of the scale – 50 = no sensation, 0 = the exact point at which what you feel transitions to pain, +50 = worst pain you can imagine.

5.) Inter-trial interval (duration 5 seconds). Next trial.

Generalisation block 2 (amplification of pain, CR2)

The Generalisation phase will be similar to the Re-acquisition phase. Participants will still be required to rate the given stimulus (using the FESTNRS); however, the stimulus delivered will be a moderate-pain US previously established in the calibration phase (Figure 5). The moderate stimulus (moderate-pain US) will be paired with all face stimuli (CS+, CS- and GSs). Also, in addition to the CS+ face image and CS- face image, three GS face images will be incorporated in this phase (these will be the same ones used in the Generalisation block 1 - behavioural response). A control face will also be shown to the participants during this phase. The control face will be a completely new face and will be paired with a moderate-pain US.

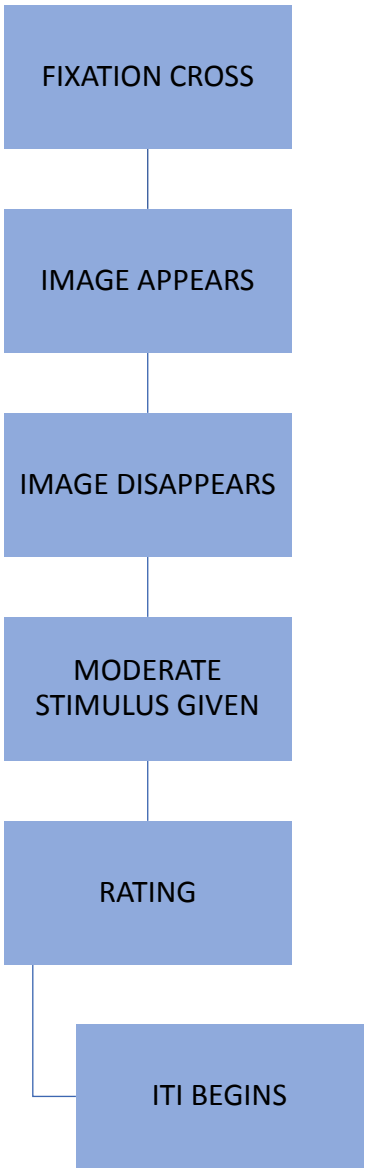


Figure 6. Generalisation of amplification of pain experimental trial procedure.

Extinction block 1

This second Extinction block will consist of 12 trials, where participants will be shown the conditioned stimuli (CS+, CS- and DS faces). No electrocutaneous stimuli (low, moderate or high) will be paired with the conditioned stimuli. Participants will not have the button press option.

Extinction block 2

Participants will be given the option of the button push and shown the conditioned stimuli

(CS+, CS- and DS faces). Participants will be told that they have unlimited button pushes. No electrocutaneous stimuli (low, moderate and high) will be given during the Extinction phase.

Blinding

Blinding of the participant will be assessed by interrogating the participant about whether they were able to identify the researchers' hypotheses. Questions will be asked such as, 'What do you think this study was investigating?' Participants will be considered blinded to the hypotheses if they are unable to identify the GSs and the role they play in the experiment. Aside from being blinded to which electrode is associated with the CS- and which is associated with the CS+, further blinding of the researcher will not be necessary, because all participant responses will be recorded by Affect, minimising reporting bias and researcher involvement. Furthermore, set instructions will be followed to minimise coaching of participants during the experiment (Attachment 2).

Contingency Awareness

Contingency awareness is the knowledge of the reinforced conditioned stimuli, that is, the knowledge that one face is paired with a high-pain stimulus and the other is face is paired with a no-pain stimulus. While controversial, there is evidence that the learning of this association is needed for classical conditioning. Contingency awareness will be assessed in two ways. First, by data analysis, where the predicted likelihood of pain rating for the CS+ will be subtracted from the CS- rating. Participants with a result greater than 7 (CS^{+} - CS^{-} > 7) will be considered contingency aware of the CS+ and CS-. A result of less than 8 (CS^{+} - CS^{-} < 8) will be indicative of contingency unawareness of the CS+ and CS-.

At the end of the procedure, participants will be given pictures of each image (CS+, CS- and GSs) and asked, 'During the experiment, how much did you associate this face with the highly painful stimulus?', with 0 = 'not at all' and 100 = 'very much'. Participants giving a rating of 80—100 for the CS+ and a rating of 0—20 for the CS- will be classified as 'contingency aware'. Any participant whose ratings are outside of these ranges will be classified as 'contingency unaware'.

We will consider the ratings that are given during the contingency assessment to provide insight into whether participants were consciously or unconsciously aware of the conditioning. However, we acknowledge that this would only provide an indication and would not be a direct test of conscious versus unconscious awareness.

Manipulation check

A two-way forced choice paradigm will be used to assess participants' ability to distinguish between the different face images used in the experiment. After the experiment, participants will be shown a series of faces in pairs, one after another. Participants will be asked, following the second face, to rate whether the two faces that they have just viewed were the same or different. Their responses will be recorded and analysed.

Questionnaires

The following questionnaires will be used to assess psychosocial factors that may impact on the findings: The Fear-Avoidance Beliefs Questionnaire (FABQ; George et al. 2006), the Catastrophising Assessment Tool (CAT; Lefebvre et al. 2002) and the Revised Neurophysiology Pain Questionnaire (Catley et al. 2013). Avoidance behaviour is a spontaneous and adaptive response to pain or fear or pain. The FABQ has been helpful for identifying individuals who are at higher risk of avoiding pain and, therefore, at higher risk of developing persistent pain. The FABQ has high internal consistency (α = 0.70—0.83), moderate test-retest reliability (r_s = 0.64—0.80) and moderate concurrent validity (ranging from r_s = 0.33—0.59; Waddell et al. 1993). The CAT is used to assess three dimensions of catastrophising – rumination, magnification and helplessness (Lefebvre et al. 2002). Catastrophising has been linked to risk of persistent pain (Keefe et al. 2004; Sullivan et al. 1995). High scores may delineate a subgroup that requires further exploration in future experiments. The Neurophysiology Pain Questionnaire will allow us to determine whether there is any difference in the findings that could be attributed to levels of pain knowledge. Several studies (Moseley et al. 2004; Van Oosterwijck et al. 2011) have demonstrated that the level of pain knowledge of people who suffer from chronic pain impacts on their pain, coping, catastrophising and other psychosocial factors (Catley et al. 2013). Although the experiment is being conducted on healthy participants, this study is investigating whether

a difference in pain knowledge influences generalisation of either the predicted likelihood of pain or amplified pain.

Proposed statistical analysis

All data will be analysed using SPSS (Version 21.0; IBM, 2012).

Normality

Data distributions will be analysed using Shapiro-Wilk tests. There is a high expectation that the predicted likelihood of pain ratings data will have a large proportion '0' ratings, causing a skewed distribution. If this is evident, then skewness will be analysed. If found to be between 1 and -1 with a non-significant *z*-score, parametric tests will be used in further analyses. If these parameters are not met, the data will be transformed and normality re-assessed. However, if after re-analysing the data its distribution is still non-normal, then non-parametric tests, equivalent to the parametric tests detailed below, will be used.

Parametric analysis

Sphericity will be assessed, where appropriate, using Mauchly's test and, if violated, then appropriate corrections will be made, such as reporting of Greenhouse-Geisser statistics (Field 2009). Bonferroni corrections will be used to minimise the risk of false positives (Type-1 error). This is a more conservative corrective measure, as it reduces the alpha value for the comparisons of interest based on the number of comparisons being made (Field 2009).

A change in the *a priori* protocol for data analysis was necessary because not all the participants used the button option. Therefore, the US expectancy ratings will be analysed as the primary outcome and the button option results will be analysed for hypotheses generation. This change in the analysis was made before the analysis was conducted.

Generalisation of US expectancy ratings

Objective one is to investigate whether US expectancy is rated higher for a CS+ than for other face stimuli, and whether the US expectancy ratings will decrease as the similarity of the face stimuli to the CS+ decrease. A one-way repeated-measures ANOVA with five levels (face images: CS+, CS- and GS1-GS3) will be conducted. Simple contrasts will be used to determine the difference between the US expectancy ratings for the CS- and the CS+ and GSs.

Contingency awareness and US expectancy

Objective two is to investigate whether contingency awareness influenced the US expectancy ratings for the different face stimuli. After allocation of the participants to aware and unaware groups, a two-way mixed ANOVA, with a between-subjects factor of group (2: aware / unaware) x within-subject factor of face images (5: CS+, GS1-3 and CS-) will be conducted.

Generalisation of amplification of pain

To investigate the amplification of pain, a paired *t*-test will be conducted for the pain ratings for the CS+ and CS-. To analyse the reliability of the three conditions (CS+ face, CS- face and control face) Chronbach's alpha will be used. To investigate generalisation of amplified pain to the GSs, a one-way ANOVA with five levels (CS+, CS- and GSs) will be conducted. Simple contrasts will be conducted to determine the difference between pain ratings of test stimuli paired with the CS-, and paired with the CS+ and GSs. We hypothesise that the pain ratings for CS+ trials will be greater than for CS- trials, and that the pain ratings to the GSs (GS1-3) will decrease as the similarity of the GS to the CS+ decreases. Moreover, we hypothesise that the pain rating for CS+ in the test phase will be greater than the rating originally given for the same electrocutaneous stimulus given during calibration.

The following questionnaires will be used to assess psychosocial factors that may impact on the findings: The Fear-Avoidance Beliefs Questionnaire (FABQ; George et al. 2006), Catastrophising Assessment Tool (CAT; Lefebvre et al. 2002) and the Revised Neurophysiology Pain Questionnaire (Catley et al. 2013). Avoidance behaviour is a spontaneous and adaptive response to pain or fear or pain. The FABQ has been helpful at identifying individuals who are at higher risk of avoiding pain and therefore at higher risk

of developing persistent pain. The FABQ has high internal consistency (r_s= 0.70—0.83), moderate test-retest reliability (r_s = 0.64—0.80) and moderate concurrent validity (r_s = 0.33—0.59; Waddell et al. 1993). The CAT is used to assess three dimensions of catastrophising – rumination, magnification and helplessness (Lefebvre et al. 2002). Catastrophising has been linked to the risk of persistent pain (Keefe et al. 2004; Sullivan et al. 1995). High scores may delineate a subgroup that require further exploration in experiments. The Neurophysiology Pain Questionnaire will allow us to determine whether there is any difference in the findings that could be attributed to levels of pain knowledge. Several studies (Moseley et al. 2004; Van Oosterwijck et al. 2011) have demonstrated that the level of pain knowledge of people who suffer from chronic pain impacts on their pain, coping, catastrophising and other psychosocial factors (Catley et al. 2013). Although the experiment is being conducted on healthy participants, this study is investigating whether a difference in pain knowledge influences generalisation of either predicted likelihood of pain or amplified pain.

Secondary analysis

Planned secondary analyses of relationships between the FABQ and CAT questionnaire results and findings will be conducted. For each questionnaire, the participant's raw scores will be used. A two-tiered approach will be used, where the top and bottom eight participant scores will be used. Analysis of the generalisation of predicted likelihood of pain ratings and pain intensity ratings for these 16 participants will be analysed and compared. The results from these analyses will be used for future hypothesis generating.

Generalisation of behavioural response (CR1)

To investigate our first objective (1.a: whether a learnt behavioural response (CR 1) is associated with the CS+ and CS-), a paired *t*-test (CS+ & CS-) will be conducted. We hypothesise that the number of times the button is pushed for the CS+ will be greater than the number of times the button is pushed for the CS-.

To investigate our second objective (1.b.: whether the learnt behavioural response (CR 1)

associated with the CS+ is modulated by images of faces of varying similarity (generalisation stimuli, GS) to the originally painful neutral face (CS+)), a one-way repeated-measures ANOVA with five levels (face images: CS+, CS- and GS1-GS3) will be conducted. Simple contrasts will be conducted to determine the difference between number of button pushes for the CS- and the CS+ and GSs. We hypothesise that there will be a greater number of button pushes for the CS+ than for the other face stimuli, and that the number will decrease as the similarity of the images to the CS+ decreases.

Contingency awareness and protective behavioural response

A two-way mixed ANOVA, with a between-subjects factor of group (2: aware / unaware) x within-subject factor of face images (8: CS+, GS1-3 and CS-) will be conducted to investigate the influence of contingency on behavioural response (number of button pushes). We will also investigate the influence of contingency awareness on time taken to push the button – for appropriate pushes (CS+ trials) only.

Planned between-group contrasts will compare the difference between the aware and unaware groups for number of button pushes (CR 1) for each face image (CS+, CS- and GS1-GS3). We hypothesise that the aware group will have a steeper generalisation gradient than the unaware group.

Extinction and protective behavioural response

To investigate the fourth objective (1.d.: whether the learnt behavioural response (CR 1) is decreased when the CS+ is no longer paired with a painful stimulus), a one-way ANOVA with three levels (face images CS+, CS- and DS) will be conducted.

Attachments

Attachment 1: Sample size calculation

Sample size: 21
***F* tests -** ANOVA: Repeated measures, within factors

Analysis:	*A priori*: Compute required sample size		
Input:	Effect size *F*	=	0.3
	α err prob	=	0.05
	Power (1-β error probability)	=	0.80
	Number of groups	=	2
	Number of measurements	=	5
	Correlation among repeated measures	=	0.5
	Nonsphericity correction ε	=	1
Output:	Noncentrality parameter *λ*	=	14.4000000
	Critical *F*	=	2.5365794
	Numerator df	=	4.0000000
	Denominator df	=	56.0000000
	Total sample size	=	21
	Actual power	=	0.8442768

Attachment 2: Scripts

Provided below are the scripts used during the experiment.

Button option

Before Acquisition phase and Generalisation phase, Block 1:

During this part of the experiment you will be given an option to push the button (**SPACE BAR**).

You have only a certain amount of button pushes so choose when to use the button carefully. The button IF PUSHED, the button can **STOP** the **painful** stimulus from occurring. It **WILL NOT** however stop the **other** stimuli from occurring. WHEN you see the text **BUTTON OPTION** displayed on your screen you can now choose whether to push it or not. IT IS your decision to whether to push the button or not. **REMEMBER** you have a **LIMITED** number of button pushes.

Before Extinction phase 2

During the experiment, you will be given several different options you can to respond to. During the experiment, you may be given an option to push a button (which is **SPACE BAR**). You will be made aware of this option before that particular part of the experiment starts. You will be altered to when you have the option to push the button when the test BUTTON OPTION is displayed on the screen. Only when the text **BUTTON OPTION** IS DISPLAYED can you push the button. During this time, you can choose whether you want to push the button or not. The button that you push is **SPACE BAR.**

Expectancy rating

During the experiment, you will be given several different options you can to respond to. Some ask you to rate your expectancy of a painful stimulus. You will be shown a horizontal line. The question above will be "HOW MUCH DO YOU EXPECT THE PAINFUL STIMULUS TO OCCUR NOW?". There will be 2 points labelled along this line. One point will be at the far-RIGHT end and will be labelled 'VERY MUCH', the other point will be at the far-LEFT end and will be labelled 'NOT AT ALL'. IF you are a 100% sure that the painful stimulus **WILL OCCUR** then you may click your mouse by the VERY MUCH side of the line. IF you are 100% sure that the painful stimulus will **NOT OCCUR** then you may click your mouse by the NOT AT ALL side of the line. REMEMBER you are rating how much you expect the painful stimulus to

occur. You can click your mouse ANYWHERE along the horizontal line. CLICK on the line that you think best rates your expectation of the painful stimulus occurrence.

Pain Intensity rating

During the experiment, you will be given several different options you can to respond to. Some ask you to rate your experience. Again, you will be shown a horizontal line. The question above will be "PLEASE RATE YOUR EXPERIENCE ON THE APPROPRIATE SIDE OF THE SCALE". There will be 3 points labelled on this line. One point will be at the far-RIGHT end and will be labelled 'WORST PAIN YOU CAN IMAGINE', in the middle of this line will be another point labelled 'THE EXACT POINT AT WHICH WHAT YOU FEEL TRANSITIONS TO PAIN' and the last point on far-LEFT end will be labelled 'NO SENSATION'. IF what you feel is not painful then you will click your mouse along the horizontal line between -50 (NO SENSATION) and 0 (THE EXACT POINT AT WHICH WHAT YOU FEEL TRANSITIONS TO PAIN'). IF what you feel is the 'THE EXACT POINT AT WHICH WHAT YOU FEEL TRANSITIONS TO PAIN' then you will click your mouse at this point on the line. IF what you feel is painful then you will click your mouse along the horizontal line between 0 'THE EXACT POINT AT WHICH WHAT YOU FEEL TRANSITIONS TO PAIN' and 50 WORST PAIN YOU CAN IMAGINE'. REMEMBER you are rating **THE FEELING OF THE SENSATION** in your hand. You can click your mouse **ANYWHERE** ALONG the horizontal line. CLICK on the line where you **think best rates the feeling you have experienced.**

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12.6. Appendix 6. Calibration protocol for Chapter 7

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Protocol description

This is a protocol for a calibration to be used in an experiment. The primary objective is as follows: To outline the calibration procedure used for the experiment in Chapter 7.

Method

This protocol will outline the calibration process used to assign stimuli that will be used throughout an experiment.

Target

For this experiment, electrocutaneous stimulation is being used. There are three stimulus intensities that need to be assigned (low sensation, medium pain, and high pain). The scale used for rating each electrical stimulus is the Fifty Either Side of Threshold Numerical Rating Scale (FESTNRS; Madden et al. Under review). A low sensation will be assigned to a rating of between -45 and -35 on the FESTNRS. Medium pain will be assigned to a rating of between 5 and 15 on the FESTNRS. High pain will be assigned to a rating of between 25 and 35 on the FESTNRS. Once established the stimuli intensities that evoked a rating of each, low sensation, medium pain and high pain, will be assigned and used through- out the experiment.

Equipment

A two digitmers (models DS7AH and DS7A) will be used to deliver the electrical stimulus. The following settings will be used: 200 milliseconds, 400 V_{max} square wave electrical stimuli.

Electrodes

The electrical stimulus will be delivered to the ventral surface of the forearm. Two types of electrodes will be used. For the low sensation, transcutaneous electrodes will be used and, for the medium and high pain stimuli, an intracutaneous electrode will be used. The transcutaneous electrodes will consist of a separate anode and cathode. The intracutaneous electrode will resemble that used in Inui and Kakigi (2012). The electrode has a small anode and cathode in the centre of a plastic platform.

Preparation

The area where the electrodes will be placed will be cleaned using an alcohol wipe and exfoliated using Nuprep preparation gel. The skin will be given sufficient time to dry following this cleaning process.

Placement

The length of the forearm will be measured from the medial condyle of the ulna to the styloid process. The first point for the low sensation electrode will be marked 40 mm proximal from the styloid process. The second point for the other low sensation electrode will be marked 40 mm from the first point. The two intracutaneous electrodes will be placed in between the two transcutaneous electrodes (Figure 1). The transcutaneous electrodes will be fixed onto the participant's forearm using adhesive stickers and will be filled with conductance gel using a blunt syringe. The intracutaneous electrodes will be fixed on the participant's forearm using hypoallergenic tape. The electrodes will be placed so that they do not come in contact with each other. Placement of the medium and high pain intracutaneous electrodes will be counterbalanced.

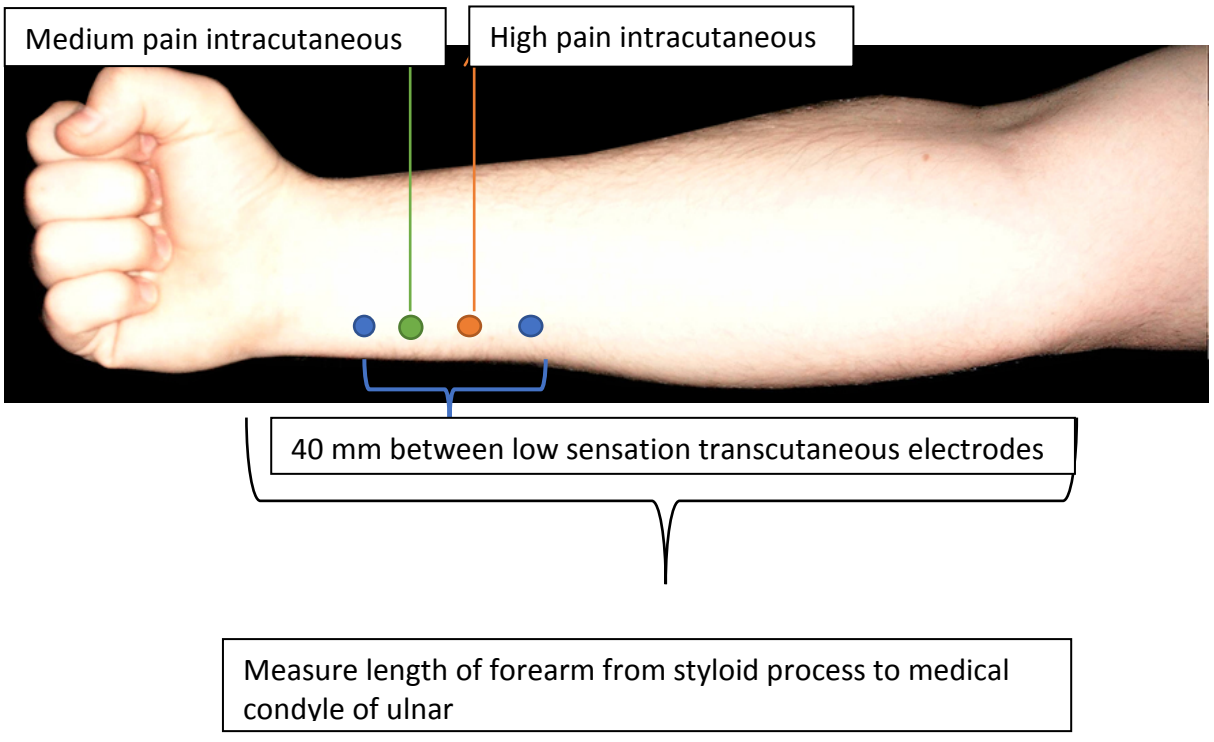


Figure 1. Electrode placement.

Practice

Before calibration begins, participants will be given several electrical stimuli at different intensities (Table 1). This will enable the participant to accommodate to the electrical stimulus and minimise the effect of affective factors in their initial ratings. During this time, any if impedance occurrs, then the electrodes will be repositioned until no impedance is shown on the Digitimer.

Table 1. Number of practice electrical stimuli given to participants.

Stimulus intensity (mA)	**Number of times delivered**
1.0	2
2.0	2
4.0	2
6.0	1
8.0	1

Calibration

Painful stimulus assignment

The procedure for the delivery of the electrical stimuli, in terms of intensity, step-wise progression and inter-pulse interval, is outlined in Table 2 (Lautenbacher & Rollman 1993).

Table 2: Calibration of painful stimuli

Electrical stimulus intensity (mA)	**Step-wise progression (mA)**	**Inter-pulse interval (sec)**
Start: 2.0	1.0	50-60
Once 9.0 reached	1.5	50-60
Once painful (intensity variable)	3.0	50-60

Once each target rating is reached, the participant will receive the same electrical stimulus intensity six times. Participant needs to rate at least four of the six stimuli within 10 points of each other on the FESTNR scale for it to become the assigned stimulus. If they rate four of the six electrical stimulations consistently higher than the targeted pain intensity rating, then the stimulus intensity will be decreased in the appropriate step-wise manner for the next trial (Larkin et al. 1986). If they rate four of the six consistently lower, then the stimulus intensity will be increased in the appropriate step-wise manner. This process will be repeated until a consistent stimulus rating (within ten points of each other) is obtained.

Low sensation stimulus assignment

The same process as outlined above will be used to assign intensity to the low sensation electrical stimulus.

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