Selectivity of conditioned fear of touch is modulated by somatosensory precision

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Abstract

Learning to initiate defenses in response to specific signals of danger is adaptive. Some chronic pain conditions, however, are characterized by widespread anxiety, avoidance, and pain consistent with a loss of defensive response specificity. Response specificity depends on ability to discriminate between safe and threatening stimuli; therefore, specificity might depend on sensory precision. This would help explain the high prevalence of chronic pain in body areas of low tactile acuity, such as the lower back, and clarify why improving sensory precision may reduce chronic pain. We compared the acquisition and generalization of fear of pain-associated vibrotactile stimuli delivered to either the hand (high tactile acuity) or the back (low tactile acuity). During acquisition, tactile stimulation at one location (CS+) predicted the noxious electrocutaneous stimulation (US), while tactile stimulation at another location (CS−) did not. Responses to three stimuli with decreasing spatial proximity to the CS+ (generalizing stimuli; GS1–3) were tested. Differential learning and generalization were compared between groups. The main outcome of fear-potentiated startle responses showed differential learning only in the hand group. Self-reported fear and expectancy confirmed differential learning and limited generalization in the hand group, and suggested undifferentiated fear and expectancy in the back group. Differences in generalization could not be inferred from the startle data. Specificity of fear responses appears to be affected by somatosensory precision. This has implications for our understanding of the role of sensory imprecision in the development of chronic pain.

Descriptors: Classical condition, Pain, Differential conditioning, Fear conditioning, Fear of touch, Generalization, Overgeneralization

Classical conditioning is a proposed mechanism responsible for the development of pain-related fear (the fear avoidance model—Lethem, Slade, Troup, & Bentley, 1983; Vlaeyen & Linton, 2000) and chronic pain itself (the imprecision hypothesis—Moseley & Vlaeyen, 2015), and the failed perceptual discrimination pathway (Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015). In these models, the cues that become associated with pain gain nociceptive-like properties and then contribute to protective behaviors and percepts. That is, previously inert conditioned stimuli (CSs), by virtue of their pairing with a noxious unconditioned stimulus (US), come to evoke preparatory conditioned responses (CRs). These CRs can be opposite or similar to those evoked by the US,

such as in fear learning. Classical conditioning is adaptive because it enables organisms to predict harm and initiate defensive responses. The extension of this learning, stimulus generalization, is also adaptive because it enables individuals to extrapolate the predictive value of one stimulus to similar stimuli (Ghirlanda & Enquist, 2003; Honig & Urcuioli, 1981). Failure to differentiate between safe and threatening cues, or overgeneralization of learning to safe stimuli, however, may lead to problems such as generalized anxiety (Baas, van Ooijen, Goudriaan, & Kenemans, 2008; Craske, Hermans, & Vansteenwegen, 2006; Grillon, 2002; Lissek & Grillon, 2010; Lissek et al., 2014; Mineka & Zinbarg, 2006), generalized pain expectancy (Meulders et al., 2014), and generalized fear of movement (Meulders, Jans, & Vlaeyen, 2015). This reduced response specificity seems to characterize some chronic pain conditions—where an increasing number of activities are avoided and an increasing array of technically harmless triggers come to evoke pain. Understanding and predicting this loss of stimulus specificity may assist in understanding the development of chronic pain.

A fundamental posit of classical conditioning theory is that specificity of conditioned responding is inversely proportional to

the extent to which stimuli can be differentiated (Ghirlanda & Enquist, 2003; Pavlov & Gantt, 1928). Thus, poorer differentiation should occur in cases of poorer acuity within the sensory system responsible for detecting that CS. Specificity of responding should therefore be lower when CSs are somatosensory signals from parts of the body where sensory precision is low—for example, the lower back, than when they are from parts of the body where sensory precision is high—for example, the hand (Goldstein, 2010; Lourens, 2014). Such differences in acuity of different body areas have been established using tests such as the two-point discrimination (TPD) threshold test, which reveals the minimum distance between two points on the skin that are able to be perceived as two points rather than one. The idea that sensory precision may modulate injury-relevant responding might assist in explaining why areas where tactile precision is most limited are also areas where chronic pain frequently develops. Chronic back, neck, and knee pain are the most prevalent, painful musculoskeletal complaints (Vos et al., 2013). Not only do these areas exhibit the lowest tactile acuities of the entire body (Goldstein, 2010; Lourens, 2014), but their respective acuity parallels their prevalence (TPD threshold: back = 41 mm, neck = 30 mm, knee = 25 mm; Lourens, 2014). In contrast, the hand exhibits high tactile acuity (TPD: 1.8–18 mm; Lourens, 2014), and is not prone to chronic pain (Vos et al., 2013).

We aimed to determine whether somatosensory precision is positively related to the specificity of conditioned fear. We hypothesized that conditioned fear would show greater specificity at the hand than at the back.

Method

Participants

Forty-eight healthy, pain-free individuals (40 females, mean age = 26 years, $SD = 9$) volunteered to participate in this study. The size of the expected effect could not be estimated; hence, a sample size was chosen in line with previous relevant studies (Lissek et al., 2010, 2014; Meulders, Jans, & Vlaeyen, 2015). Participants were recruited using flyers, social media (via bodyinmind.org), and word of mouth. They were reimbursed AU$20 for their participation. Exclusion criteria included self-reported current pain, history of chronic pain, or any self-reported psychological or neurological impairment. Participants were not informed about the experimental stimulus contingencies or the hypotheses. The protocol was conducted at the University of South Australia and approved by the Institutional Ethics Committee (Protocol #0000031100).

Stimulus Material

Painful electrocutaneous stimuli (pain-USs; 20-ms bursts with instantaneous rise and fall) were delivered using two Digitimer DS700 electrical stimulators (Digitimer Ltd., Welwyn Garden City, UK). Vibrotactile stimuli (CSs and GSs) were delivered by five 10-mm, 3V, off-centre electric motors (tactors). Tactors were set to vibrate at a strong, nonpainful intensity at approximately 220 Hz. The tactors were attached to the skin using a double-sided adhesive sticker. The anatomical location (back or hand) and spatial orientation of the tactors were counterbalanced among participants according to a prerandomized order. Tactors were equidistant (8 cm) from the pain-US and spaced equally (2 cm apart) in an identical arrangement for both back and hand (Figure 1). This arrangement was chosen to enable spatially distinct conditioned

stimuli while maintaining a close, clinically plausible relationship between tactile and nociceptive signals. Because generalization can only be evaluated if stimuli are perceptually distinct, adequate spacing of stimuli was critical and carefully chosen during pilot testing. All CSs and GSs in acquisition and generalization were presented for 5 s, and, when reinforced, pain-USs were presented immediately after.

The pain-US intensity was individually calibrated using a staircase method with a single ascending run of 0.1 mA increments. During this procedure, the intensity of the stimulus was rated using a 0–10 numerical rating scale (NRS) where 0 = *no sensation at all*, 1 = *you feel something but this is not painful, it is merely a sensation*, 2 = *you feel a strong sensation, but it is not painful*, 3 = *starts to be painful, but is a very mild pain*, 8 = *significantly painful and demanding some effort to tolerate*, and 10 = *the worst imaginable pain*. This scale was used for calibration because it distinguishes between strong sensations and painful sensations. We have used this scale and similar scales previously to ensure that electrocutaneous stimuli are truly painful (Harvie et al., 2016; Meulders, Harvie, Moseley, & Vlaeyen, 2015; Meulders, Vansteenwegen, & Vlaeyen, 2012). The stimulus intensity (mA) corresponding to an 8 on this NRS was used as the pain-US.

Manipulation Check

Stimulus generalization is a process by which stimuli that share features with the conditioned stimulus, but which are perceptibly distinct, can also elicit conditioned responses. Therefore, to show a generalization effect it was essential that all vibrotactile stimuli were perceptibly different. To test this, subjects underwent a forced-choice differentiation test in which they were exposed to adjacent pairs of stimuli, in order to determine if they could be distinguished. Prior to testing each pair, the experimenter delivered vibrotactile stimulation at each location and verbally labeled them *a* and *b*. On further trials, the participant was asked to choose whether (s)he felt stimulus a or b. This was repeated five times in semirandom order for each adjacent stimulus pair. Scoring greater than 50% on each adjacent pair was required for their classification as perceptibly different and for the participant's entry into the study.

Experimental Design

We used a differential classical conditioning paradigm with responses to the conditioning and generalization stimuli as the within-subject factors, and group (back vs. hand) as a between-subjects factor. Participants were randomly allocated to the back group or the hand group. The experiment consisted of two phases: the acquisition and the generalization phases, each containing two blocks. During the acquisition phase, a vibrotactile stimulation at one location (CS+) was always followed by a high intensity electrocutaneous stimulus (pain-US); vibrotactile stimulation at another location was not (CS−). During generalization, three intermediate stimulus locations were stimulated, and we tested the spreading of conditioned fear responses to these generalization stimuli (GS1, GS2, GS3).

During each of the two acquisition blocks, the CS+ and CS− were presented six times each. During the two generalization blocks, each of the CSs and GSs were presented three times (see Table 1). Stimuli were delivered according to six preselected semirandomized sequences. The acquisition stimuli were randomized within each block, with the restriction that no stimulus type could
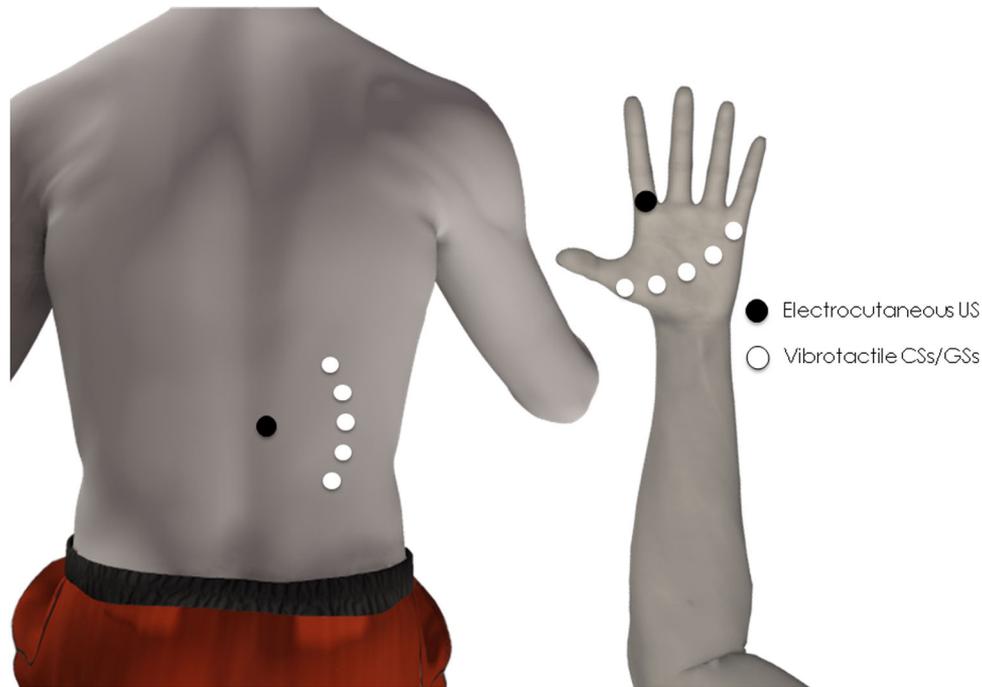
Figure 1. Experimental setup showing the placement of the electrocutaneous pain-US (black dot) and vibrotactile stimuli (white dots) for the back and hand group. The vibrotactile stimuli (CS+, GS1–3, CS−) were orientated either top to bottom or vice versa at the back, and lateral to medial or vice versa at the hand. Which stimulus location served as the CS+ or the CS− was counterbalanced across participants.

be presented in more than three consecutive trials. The generalization stimuli were also randomized within each block. Participants were allocated to one of the six sequences according to a prerandomized order. During reinforced trials, the 20-ms burst of electrocutaneous stimulation occurred at the offset of vibrotactile stimulation. All CS+ stimuli were reinforced across all phases and blocks. Vibrotactile stimuli were presented for a duration of 5 s, separated by a 12-s intertrial interval (ITI).

Dependent Measures

Primary outcome. Conditioned fear was measured through its modulating effect on physiological reactivity. The eyeblink startle response is a component of a whole body defensive physiological (startle) response and is triggered by startle-evoking stimuli, such as a sudden noise. The magnitude of this response is amplified by defensive states (such as those induced by a CS+) and can therefore be quantified by calculating a change in startle magnitude relative to a control stimulus (CS−). In this study, an acoustic startle probe was used to elicit the startle response during the CS/GS tactile stimulations. The acoustic startle probe was a 100 dBA, 50-ms burst of white noise with instantaneous rise time presented in both ears through headphones (Sennheisser HD280). In order to reduce the predictability of the startle probe, it was delivered at either 2.25, 2.5, or 2.75 s after onset of the tactile stimulus according to a prerandomized sequence. Startle responses were recorded using electrodes below the left eye. Orbicularis oculi electromyographic activity (EMG) was recorded with gold-plated surface electrodes (10-mm diameter; Compumedics Ltd.) filled with conductive gel (Quik-Gel, Compumedics Ltd.). The skin was prepared using exfoliating cream to reduce skin-electrode resistance before placement of the electrodes beneath the left eye and mastoid according to accepted site specifications (Blumenthal et al., 2005). The raw signal was amplified using a SynAmpsRT amplifier and recorded using NeuroScan 4.5 (Compumedics Ltd.) with a bandwidth of 30 Hz to 1 kHz, a sampling rate of 10,000 Hz, and a notch filter at 50 Hz to eliminate electrical artifact.

Secondary variables. In order to assist the interpretation of the physiological data, self-reported fear and pain-US expectancy associated with each vibrotactile CS/GS was assessed retrospectively after the entire experiment. Fear ratings were acquired by asking participants to rate "How afraid were you when tactor 1 was vibrating?" This was then repeated for tactors 2–5. For half of the participants, tactor 1 was the CS+; for the other half, it was the CS−. This was done to eliminate an absolute order effect across the sample. During this question, vibrators 1–5 were identified to the participants by individually touching the respective locations. Ratings were given on a 0–100 mm visual analog scale (VAS) with the anchors *not at all afraid* and *very afraid*. In the same way, pain-US expectancy for each stimulus was rated by asking the participant "To what extent were you expecting the electrocutaneous stimulus to come after tactor 1, 2, 3, 4, 5 was vibrating?" Ratings

Table 1. *Experimental Design*

Habituation	Acquisition	Generalization	Exploration
8 × startle probes	12 × CS+	6 × CS+	12 CS+
	12 × CS−	6 × GS1	9 GS1*
		6 × GS2	9 GS2*
		6 × GS3	9 GS3*
		6 × CS−	9 CS−*

Note. Stimulus presentations during acquisition and generalization were divided into two blocks with equal number of stimuli. All CS+ stimuli were reinforced.
*In the exploration phase, the CS+ was always reinforced, while the GSs and CS− were reinforced three times each.

were made on a 0–100 mm VAS with the anchors *not at all expecting the electrocutaneous stimulus* and *very much expecting the electrocutaneous stimulus*.

During the experiment, participants were asked to rate pain intensity and unpleasantness for each pain-US immediately after reinforcement, during the ITI. This enabled us to continuously check whether the calibration of the pain-US was successful, to monitor possible habituation/sensitization effects, and to analyze the effect of US painfulness on the primary outcome. In addition, this enabled seamless transition to the exploratory phase (see below), where pain-US ratings were critical. Participants recorded their pain intensity ratings on a 100-mm VAS where 0 mm = *no pain* and 100 mm = *worst imaginable pain*.

Exploratory data. A possible role for classical conditioning and generalization of pain and hyperalgesic responses has recently been proposed (Moseley & Vlaeyen, 2015). Following this hypothesis, the ability of tactile CSs to modulate pain was recently confirmed in our lab (Harvie et al., 2016); however, tactile GSs have yet to be explored. Therefore, in a postgeneralization phase, each stimulus (CS+/GS1–3/CS−) was paired with the pain-US in order to explore the possibility of generalization of a conditioned hyperalgesic effect. No startle probes were used in this phase, and thus only pain intensity ratings were collected. This was considered exploratory because it was thought that, by this stage of the experiment, the nonreinforcement during the generalization phase would likely have extinguished any generalization effect. Nonetheless, we utilized this opportunity to look for a potential trend that could be more thoroughly investigated in later studies.

Protocol

No information about the experimental contingencies or hypotheses was given. After being informed of the broad nature of the study, participants gave signed informed consent. The electrodes and tactors were then attached (Figure 1) and the pain-US calibrated (following the previously described calibration procedure).

Software and apparatus. The experiment was executed using a custom-built, Arduino-based module, which controlled the timing and intensity of the two tactors and triggered the two Digitimer DS700 electrical stimulators via TTL signaling. The prerandomized stimulus presentation sequences were uploaded to the Arduino module using customized LabVIEW software. Electrodes and tactors were placed on the left arm, which was positioned behind a screen to eliminate visual feedback about the location of the various stimuli. During the procedure, the experimenter was in a separate room and did not interact with the participant.

Response definition of the fear-potentiated startle. Offline processing was undertaken using Curry Neuroimaging Suite 7. The EMG signal was rectified, and a 55 Hz low-pass filter applied. A baseline correction was applied, and the EMG trace smoothed. Following these processing steps, we calculated the peak amplitude of each response, defined as the maximum of the response curve within 20–175 ms after the startle probe onset. All startle waveforms were visually inspected, and trials containing technical abnormalities and artifacts were eliminated and recorded for reporting purposes. The raw scores were transformed to z scores to account for interindividual differences in startle reactivity prior to analysis.

Statistical Analysis Overview

A series of repeated measures analyses of variance (ANOVAs) was used to examine the differences between the back and hand groups with respect to differential fear learning and fear generalization. The EMG data were analyzed by block because learning and extinction theories imply that differential learning and generalization would be best observed at the end of acquisition and beginning of generalization (where the influence of extinction effects is most limited). Because we had clear a priori hypotheses, we further analyzed the data using planned comparisons. In order to correct for multiple comparisons and reduce the probability of a Type I error, we used Holm-Bonferroni corrections. This was chosen over the Bonferroni method, because we considered it to be too conservative and can inflate the likelihood of a Type II error (Perneger, 1998). The effect size indication η_p^2 is reported for ANOVA effects.

Alpha was set at $p = .05$ for all ANOVAs. Where the assumption of sphericity was violated, Greenhouse-Geisser corrected p values and associated epsilon (ε) values were reported, along with the uncorrected degrees of freedom. Effect sizes—partial eta-squared (η_p^2)—were interpreted using Cohen's guidelines (.01 = small, .059 = medium, and .138 = large; Cohen, 1988). All ANOVAs were conducted using an alpha level of .05. Analyses with more than two levels used Greenhouse-Geisser corrected degrees of freedom to determine significance, though uncorrected degrees of freedom are reported.

Results

Subjects

Forty-eight healthy people were allocated to either the back group (18 females, 6 men) or the hand group (22 females, 2 men). T tests for independent samples confirmed there were no group differences with respect to pain catastrophizing (Pain Catastrophizing Scale; Sullivan, Bishop, & Pivik, 1995)—back: $M = 19.65$, $SD = 9.77$, hand: $M = 17.88$, $SD = 8.65$; stress (Depression Anxiety Stress Scale; Lovibond & Lovibond, 1995)—back: $M = 6.9$, $SD = 6.33$, hand: $M = 10.16$, $SD = 6.79$; anxiety (Depression Anxiety Stress Scale; Lovibond & Lovibond, 1995)—back: $M = 7.48$, $SD = 7.41$, hand: $M = 2.91$, $SD = 3.52$; depression (Depression Anxiety Stress Scale; Lovibond & Lovibond, 1995)—back: $M = 3.34$, $SD = 4.20$, hand: $M = 6.80$, $SD = 9.44$; and age—back: $M = 25.28$, $SD = 7.12$, hand: $M = 27.08$, $SD = 10.91$) ($p > .05$).

Manipulation Checks

CS discriminability and baseline pain-US intensity. All subjects differentiated between adjacent stimuli at better-than-chance consistency, confirming that the CS and GS stimuli were perceptually different. The chosen calibrated intensity of the pain-US was equal for both groups with respect to the applied current (back: $M = 88.87$ mA, $SD = 41.68$, hand: $M = 78.32$ mA, $SD = 45.93$; $t(46) = 0.83$, $p = .41$) and the perceived intensity—with all subjects reaching the target 8/10 intensity during calibration.

Retrospective pain-US expectancy. Retrospective pain-US expectancy ratings were analyzed using a 2 × 5 Group (back/hand) × Stimulus Type (CS+/GS1–3/CS−) ANOVA. This yielded a significant main effect of stimulus type, $F(4,184) = 5.90$, $p < .01$, $\eta_p^2 = .114$, $\varepsilon = .53$; however, the Stimulus × Group interaction, $F(4,184) = 0.66$, $p = 0.53$, $\eta_p^2 = .01$, was not significant (Figure 2).
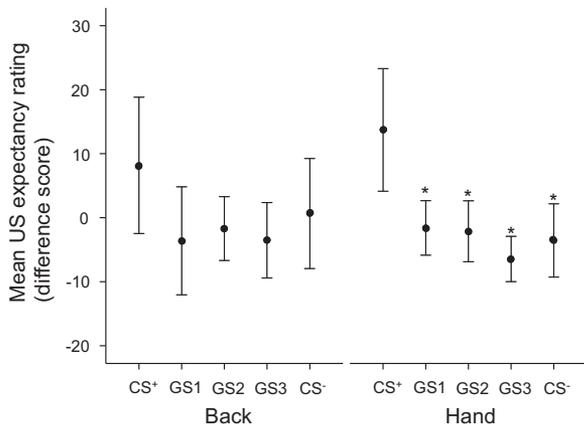
Figure 2. Retrospective pain-US expectancy ratings normalized as the difference between the mean expectancy rating across all stimulus types and the expectancy rating for each stimulus. Bars represent 95% CI. *Stimulus ratings are significantly different to the CS+ rating ($p < .05$).

Notwithstanding the absence of the two-way interaction, we further tested generalization within each group using planned comparisons. In the hand group, there was a large effect of stimulus type, $F(4,96) = 6.66$, $p < .01$, $\eta_p^2 = .22$, $\varepsilon = .50$, with Holm-Bonferroni corrected comparisons showing that the CS+ evoked greater expectancy than all other stimuli, $p < .05$. In the back group, however, expectancy ratings were not affected by stimulus type, $F(4,88) = 1.31$, $p = .28$, $\eta_p^2 = .06$, $\varepsilon = .49$.

Retrospective fear ratings. Retrospective fear ratings were analyzed using a 2×5 Group (back/hand) $\times$ Stimulus Type (CS+/GS1–3/CS−) ANOVA. This yielded a significant main effect of stimulus type, $F(4,184) = 4.97$, $p = .004$, $\eta_p^2 = .097$, $\varepsilon = .652$, and no interaction with group, $F(4,184) = 0.67$, $p = .55$ (Figure 3). We further tested the effect of conditioning on self-reported fear using planned comparisons within each group. In the hand group, there was a large effect of stimulus type, $F(4,96) = 4.60$, $p = .008$, $\eta_p^2 = .161$, $\varepsilon = .657$, with Holm-Bonferroni corrected comparisons showing that the CS+ was associated with greater fear relative to the GS3 and CS− ($p = .005$ and .006, respectively), but not greater than the GS1 and 2 ($p = .05$). In the back group, however, fear rat-
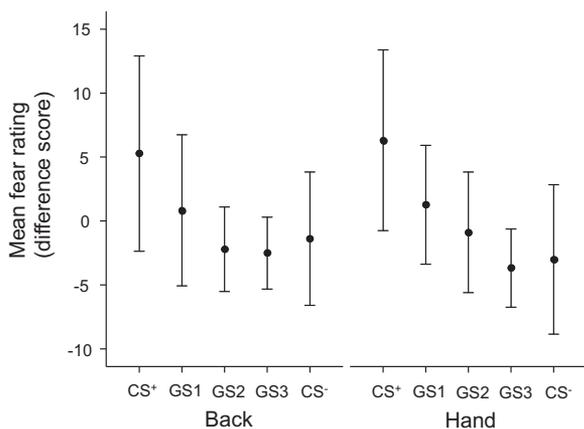

Figure 3. Retrospective fear ratings normalized as the difference between the mean fear rating across all stimulus types and the fear rating for each stimulus. Error bars represent 95% CI. *Stimulus ratings are significantly different to the CS+ rating ($p < .05$).
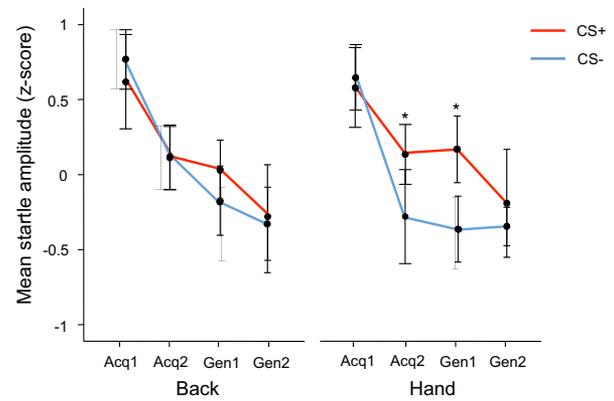

Figure 4. EMG startle responses for the CS+ and CS− stimuli for the back and the hand groups across the four study blocks. Error bars represent 95% CI. Acq1 and Acq2 represent the two acquisition phase blocks, Gen1 and Gen2 represent the two generalization phase blocks. Error bars represent 95% CI. *Significantly different in CS+ versus CS− responses within that phase ($p < .05$).

ings were not affected by stimulus type, $F(4,88) = 1.20$, $p = .312$, $\eta_p^2 = .052$, $\varepsilon = .539$.

Testing the Primary Hypotheses

Startle data for all 48 participants were included. Eight percent of trials did not meet our criteria for inclusion and were eliminated. The reasons for exclusion were nonresponse and temporary signal loss (2.5%), electrical artifact (3%), or being more than 3 SD away from the mean (2.5%). Thus, the final analysis was undertaken on the remaining 92% (2,893) of trials.

Acquisition. Acquisition of differential fear learning in the startle responses was analyzed using a $2 \times 2 \times 2$ Group (back/hand) $\times$ Stimulus Type (CS+/CS−) $\times$ Block (ACQ1/ACQ2) repeated measures ANOVA. While there was no overall effect of stimulus type, $F(1,46) = 2.38$, $p = .13$, $\eta_p^2 = .05$, there was a significant Stimulus Type $\times$ Block $\times$ Body Site interaction $F(1,46) = 4.55$, $p = .04$, $\eta_p^2 = .09$ (Figure 4). Following this finding and in accordance with our a priori plan, we subsequently compared CS+/CS− within each block for each group. During the first acquisition block, startle responses were not different for the CS+ versus the CS− at either the back, $t(22) = -0.95$, $p = .35$, or hand, $t(24) = -0.6$, $p = .6$. During the second acquisition block, however, a differential effect was evident at the hand, $t(24) = 2.4$, $p = .024$ while still absent for the back, $t(22) = 0.47$, $p = .96$.

Generalization. Generalization phase startle responses were analyzed using a $2 \times 5 \times 2$ Group (back/hand) $\times$ Stimulus Type (CS+/GS1–3/CS−) $\times$ Block (GEN1/GEN2) repeated measures ANOVA. Within generalization, an overall effect of stimulus type, $F(4,44) = 3.15$, $p = .02$, $\eta_p^2 = .07$, block $F(1,44) = 6.24$, $p = .02$, $\eta_p^2 = .12$, and a Stimulus Type $\times$ Block $\times$ Group interaction, $F(4,44) = 2.5$, $p < .05$, $\eta_p^2 = .05$, were revealed (Figure 5). Planned comparisons within each group and block were subsequently undertaken. For the hand group, startle responses for the CS+ were significantly different to the most distant two stimuli ($p = .02$ and $p < .01$ for the GS3 and CS−, respectively) but not different to the closest two stimuli ($p = .07$ for the GS1 and GS2) during the first generalization block. For the back, however, only the CS+ and
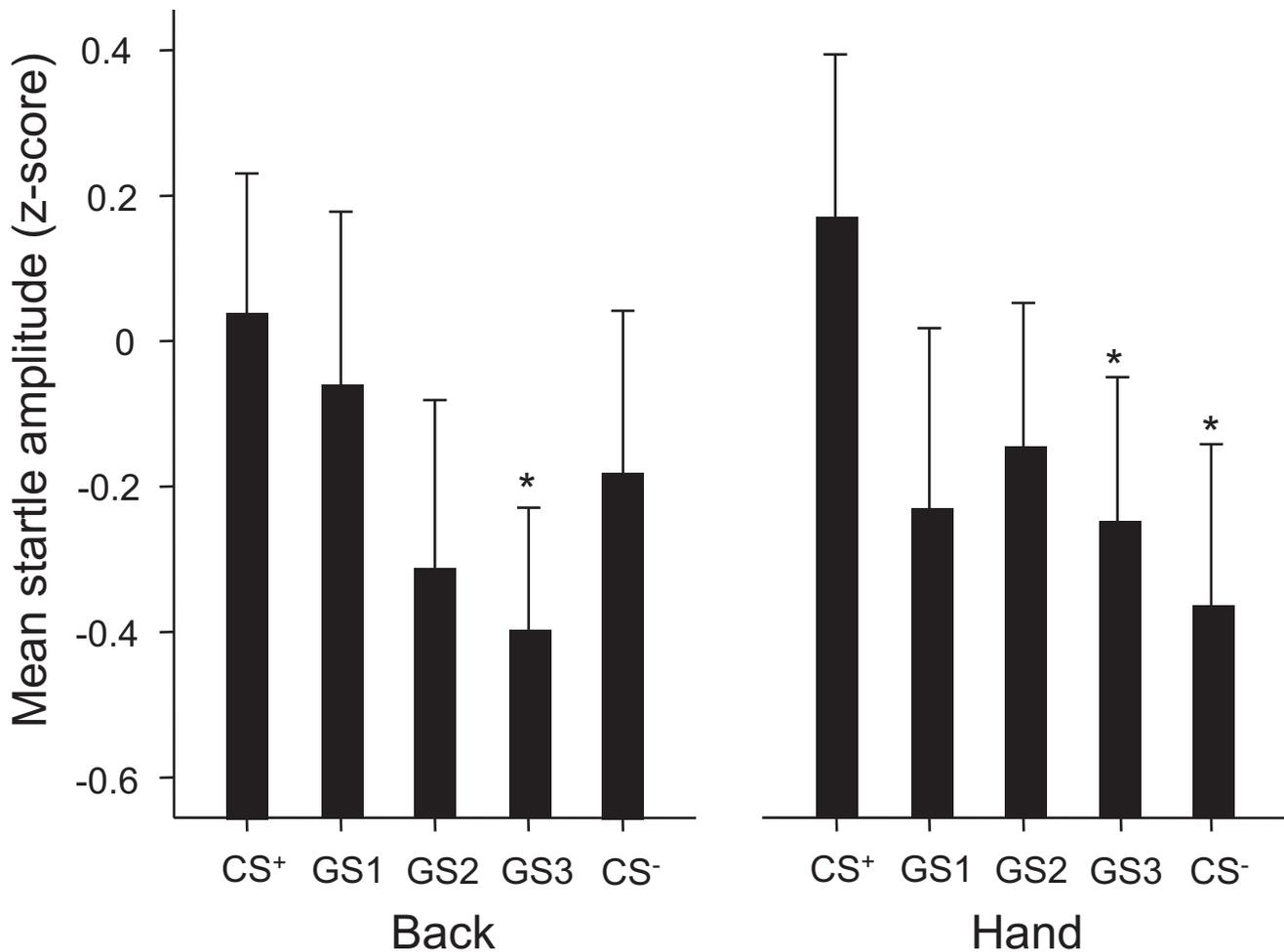
Figure 5. EMG startle responses for the CS+, GS, and CS− stimuli for the back and the hand groups in generalization block 1. Error bars represent 95% CI. *Significantly different from the CS+.

GS3 were significantly different ($p < .01$). No effect of stimulus was evident during the second generalization block.

Exploratory Analysis

In order to explore the effect of tactile stimuli on US-evoked pain, a 2×5 Group (back/hand) $\times$ Stimulus Type (CS+/GS1–3/CS−) repeated measures ANOVA was undertaken on the pain ratings in the postgeneralization, exploratory phase. No significant effects emerged ($p > .05$).

Discussion

In this study, we investigated whether somatosensory precision is positively related to the specificity of conditioned fear responses. We hypothesized that conditioned fear responses would show greater specificity at the hand, where sensory precision is high, than at the back, where sensory precision is low. The hypothesis was supported in that startle responses showed differential learning only in the hand group. Self-reported fear and pain-US expectancy ratings confirmed differential learning and limited generalization in the hand group, and suggested undifferentiated fear and pain-US expectancy in the back group. Differences in generalization could not be inferred from the EMG startle data because there was no reliable acquisition effect in the back group.

Discriminating Threatening and Nonthreatening Cues

Participants in the hand group demonstrated differential fear responding to the CS+ tactile stimulus relative to the CS− stimulus, as evidenced by the higher startle amplitudes to the CS+ relative to the CS−, which was apparent by the second acquisition block. Differential responses were not apparent in the back group. That the ANOVA approached significance ($p = .096$) raises the possibility that learning was delayed, rather than absent. Fear and pain-US expectancy ratings were consistent with the EMG data in that the hand group, but not the back group, reported greater fear and pain-US expectancy during the CS+ than during the CS−. The EMG, fear, and expectancy data support the hypothesis that differential learning is positively related to tactile sensory precision.

Stimulus Generalization

Preexperiment manipulation testing revealed that all subjects were able to differentiate between adjacent tactile stimuli, confirming that responses to GS that were greater than responses to the CS− could be considered generalization—not merely a product of being indistinguishable from the CS+. Notably, this was not designed to support the established differences in acuity between back and hand, and it was insensitive to do so because we specifically designed the stimulus separation for distinguishability. A similar procedure with closer

stimuli, however, would likely have confirmed the differences in acuity that have been shown with TPD tests and other procedures designed for this purpose (Goldstein, 2010; Lourens, 2014).

In the hand group, there was evidence of generalization of fear to the GS1 and GS2 in the EMG and fear rating data, in that responses to these stimuli in the first generalization block were not significantly different to the CS+. While not statistically significant, the GS2 related to slightly greater startle responses than the GS1. This deviation from the expected gradient merits some consideration. Given the lesser tactile acuity in the area at which GS2 was located (Lourens, 2014), one might predict a greater generalization at GS2 than at GS1. Visual inspection of the data raises the possibility that we were underpowered to detect this effect, although clearly further work is required to determine this.

Given the failure of differential learning consistently shown across the EMG and rating data, one would expect the back group to show undifferentiated responding among all CS and GS stimuli. This was true for the fear and pain-US expectancy data; however, an anomalous CS+/GS3 difference was found in the EMG data. We are unable to interpret this finding, but speculate that it either reflects a false-positive finding or the presence of distinct subgroups of participants who respond in different ways. Nonetheless, because generalization depends on differential learning, which was not shown for the back group, this study cannot ascertain whether generalization is modulated by somatosensory precision. We suggest that a further study using CSs at greater separations, or the use of a longer learning phase, might cast light on this issue.

Clinical Relevance

Not only does chronic pain seem to develop more commonly in areas of the body where sensory precision is low (Vos et al., 2013), but, in addition, people with chronic pain show reduced precision in tests of sensory acuity (i.e., tactile and proprioceptive), which seem to correspond to the precision of cortical representations (Catley, O'Connell, Berryman, Ayhan, & Moseley, 2014; Flor, 2014; Moseley & Flor, 2012; Wand et al., 2011). The idea that imprecision may result in reduced specificity of defensive responses may help to explain the spread of behavioral and perceptual symptoms seen in common chronic pain conditions, such as chronic widespread pain, fibromyalgia syndrome, and nonspecific low back pain. Recently, two theories have been proposed outlining how reduced sensory/cortical precision and reduced discriminative capacity might modulate the outcome of classical conditioning and contribute to the sensory/perceptual components of chronic pain. Moseley and Vlaeyen (2015) contend that pain might become a CR, and that difficulty in discriminating CSs from other stimuli might result in a greater array of stimuli evoking pain. Zaman et al. (2015) propose that CSs, which can become aversive, unpleasant, and more intense through fear learning, are becoming difficult to distinguish from painful USs. When there is reduced sensory acuity, this perceptual discrimination difficulty might be compounded, resulting in a greater number of painful percepts (Zaman et al., 2015). Of course, other factors also influence selectivity of conditioned responses, such as attention/cognitive variables and the impact of verbal and vicarious learning pathways. Based on these theoretical frameworks, a range of therapeutic approaches may be useful in making defensive responses more specific and thus treating chronic pain and its associated symptoms. These may include sensory acuity training (Flor, 2012; Flor, Denke, Schaefer, & Grüsser, 2001; Moseley, Zalucki, & Wiech, 2008; Moseley & Wiech, 2009), exposure/extinction procedures (Boersma et al., 2004; Bowering et al., 2013; Linton et al., 2008; Vlaeyen, de Jong, Geilen,

Heuts, & van Breukelen, 2001, 2002), and cognitive approaches (Moseley, 2003, 2004). Notably, while the current study supported a role for sensory acuity in modulating the specificity of defensive fear responding, the exploratory phase does not support a role for sensory acuity in modulating the specificity of pain or hyperalgesic responses. However, the lack of effect may be explained by extinction of differential learning that was demonstrated in the EMG data prior to commencement of the exploratory phase.

Limitations

One of the main limitations of the current study was with respect to the timing and method used to gauge the propositional outcome of conditioning. Although retrospectively assessing self-reported fear and expectation may have protected the startle data from the effects of experimental demand (Boddez et al., 2013), offline ratings do not necessarily relate well to online measures (Grillon et al., 2008). Furthermore, the experimenter touched and described the location relating to each question, rather than delivering a vibration. While this method may have accurately gauged the spatial aspects of learning, it is possible that the vibrotactile stimulation produced different nonspatial qualities at different locations and therefore may not have precisely reflected learning. Also, ratings for each stimulus were acquired sequentially, beginning either at the CS+ or CS−. While this counterbalancing reduced the possibility of a systematic bias toward the CS+ or CS−, a randomized questioning would have diminished the likelihood of an order effect not accounted for by counterbalancing. Finally, for the self-report data, only the planned comparisons were able to elucidate the differences between groups. Therefore, we suggest that additional research is needed to further evaluate the limited evidence brought forward by the current study. Despite the limitation in the self-report data, they broadly paralleled the physiological data, thereby providing support for the main findings.

Because of a lack of differential responding in the back group, we were unable to compare generalization between groups with respect to the primary outcome. We suggest that future studies employ a design with more spatially distinct CSs and/or with a greater number of acquisition trials. In order to accommodate a larger stimulus array, however, the arrangement of stimuli on the hand will have to be reconsidered or moved to a different body region.

A further limitation of the current study was the lack of startle probes in the ITI, which would have further assisted interpretation and analysis of hand versus back group differences. These were not used in order to limit the number of probes and reduce the influence of habituation on intrastimulus probes. The ITI information, however, would have provided information that might have further assisted interpretation. For example, three main scenarios were likely with regard to predictive learning and ITI fear responses: (1) individuals might learn the predictors of pain—resulting in discrete fear responses to those predictors; (2) individuals might learn that vibrotactile stimulation is necessary for stimulation, but not which vibrotactile stimulus is predictive of the pain-US—resulting in fear responses during all vibrotactile stimulation but not the intertrial period; or (3) individuals might not learn to predict painful stimulation at all—resulting in contextual fear responding. The ITI data might have assisted in differentiating between the latter two possibilities within and between groups, and therefore should be considered in future studies.

Conclusion

This study shows that the specificity of conditioned fear responding is positively related to somatosensory precision,

in that greater differential responding was shown at the hand relative to the back. Whether generalization is related to somatosensory precision remains to be determined. Our results have clear implications for our understanding of the role of sensory imprecision in the development of chronic pain conditions that are associated with loss of sensory and cortical precision and for conditions that develop in areas of low sensory precision.

References

Baas J. M., van Ooijen L., Goudriaan A., & Kenemans J. L. (2008). Failure to condition to a cue is associated with sustained contextual fear. *Acta Psychologica*, *127*, 581–592. doi: 10.1016/j.actpsy.2007.09.009

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*, 1–15.

Boddez, Y., Baeyens, F., Luyten, L., Vansteenwegen, D., Hermans, D., & Beckers, T. (2013). Rating data are underrated: Validity of US expectancy in human fear conditioning. *Journal of Behavior Therapy and Experimental Psychiatry*, *44*, 201–206. doi: 10.1016/j.jbtep.2012.08.003

Boersma, K., Linton, S., Overmeer, T., Jansson, M., Vlaeyen, J., & de Jong, J. (2004). Lowering fear-avoidance and enhancing function through exposure in vivo. A multiple baseline study across six patients with back pain. *PAIN*, *108*, 8–16. doi: 10.1016/j.pain.2003.03.001

Bowering, K. J., O'Connell, N. E., Tabor, A., Catley, M. J., Leake, H. B., Moseley, G. L., & Stanton, T. R. (2013). The effects of graded motor imagery and its components on chronic pain: A systematic review and meta-analysis. *Journal of Pain*, *14*, 3–13. doi: 10.1016/j.jpain.2012.09.007

Catley, M. J., O'Connell, N. E., Berryman, C., Ayhan, F. F., & Moseley, G. L. (2014). Is tactile acuity altered in people with chronic pain? A systematic review and meta-analysis. *Journal of Pain*, *15*, 985–1000. doi: 10.1016/j.jpain.2014.06.009

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. New York, NY: Psychology Press.

Craske, M. G., Hermans, D. E., & Vansteenwegen, D. E. (2006). *Fear and learning: From basic processes to clinical implications*. Worcester, MA: American Psychological Association. doi: 10.1037/11474-000

Flor, H. (2012). New developments in the understanding and management of persistent pain. *Current Opinion in Psychiatry*, *25*, 109–113. doi: 10.1097/YCO.0b013e3283503510

Flor, H. (2014). Role of cortical reorganization in the rehabilitation of chronic pain. In W. Jensen, O. K. Andersen, & M. Akay (Eds.), *Replace, repair, restore, relieve–Bridging clinical and engineering solutions in neurorehabilitation* (pp. 1–2). New York, NY: Springer International Publishing. doi: 10.1007/978-3-319-08072-7_1

Flor, H., Denke, C., Schaefer, M., & Grüsser, S. (2001). Effect of sensory discrimination training on cortical reorganisation and phantom limb pain. *Lancet*, *357*, 1763–1764. doi: 10.1016/S0140-6736(00)04890-X

Ghirlanda, S., & Enquist, M. (2003). A century of generalization. *Animal Behaviour*, *66*, 15–36. doi: 10.1006/anbe.2003.2174

Goldstein, E. B. (2010). *Sensation and perception* (8th ed.). Belmont, CA: Wadsworth, Cengage Learning.

Grillon, C. (2002). Startle reactivity and anxiety disorders: Aversive conditioning, context, and neurobiology. *Biological Psychiatry*, *52*, 958–975. doi: 10.1016/S0006-3223(02)01665-7

Grillon, C., Lissek, S., Rabin, S., McDowell, D., Dvir, S., & Pine, D. S. (2008). Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiologic marker of panic disorder. *American Journal of Psychiatry*, *165*, 898–904. doi: 10.1176/appi.ajp.2007.07101581

Harvie, D. S., Meulders, A., Victoria, J. M., Hillier, S. L., Peto, D. K., Brinkworth, R., & Moseley, G. L. (2016). When touch predicts pain: Predictive tactile cues modulate perceived intensity of painful stimulation independent of expectancy. *Scandinavian Journal of Pain*, *11*, 11–18. doi: 10.1016/j.sjpain.2015.09.007

Honig, W. K., & Urcuioli, P. J. (1981). The legacy of Guttman and Kalish (1956): 25 years of research on stimulus generalization. *Journal of the Experimental Analysis of Behavior*, *36*, 405–445. doi: 10.1901/jeab.1981.36-405

Lethem, J., Slade, P., Troup, J., & Bentley, G. (1983). Outline of a fear-avoidance model of exaggerated pain perception—I. *Behaviour Research and Therapy*, *21*, 401–408. doi: 10.1016/0005-7967(83)90009-8

Linton, S. J., Boersma, K., Jansson, M., Overmeer, T., Lindblom, K., & Vlaeyen, J. W. (2008). A randomized controlled trial of exposure in vivo for patients with spinal pain reporting fear of work-related activities. *European Journal of Pain*, *12*, 722–730. doi: 10.1016/j.ejpain.2007.11.001

Lissek, S., & Grillon, C. (2010). Overgeneralization of conditioned fear in the anxiety disorders. *Journal of Psychology*, *218*, 146–148. doi: 10.1027/0044-3409/a000022

Lissek, S., Kaczkurkin, A. N., Rabin, S., Geraci, M., Pine, D. S., & Grillon, C. (2014). Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. *Biological Psychiatry*, *75*, 909–915. doi: 10.1016/j.biopsych.2013.07.025

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychology*. *167*, 47–55. doi: 10.1176/appi.ajp.2009.09030410

Lourens, E. (2014). *Normative values for two-point discrimination in healthy adults* (Honour's thesis, The University of South Australia). Retrieved from http://search.library.unisa.edu.au/media/researcharchive/open/9915960266401831/53112422410001831

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney. Australia: Psychology Foundation.

Meulders, A., Harvie, D. S., Bowering, J. K., Caragianis, S., Vlaeyen, J. W. S., & Moseley, G. L. (2014). Contingency learning deficits and generalization in chronic unilateral hand pain patients. *Journal of Pain*, *15*, 1046–1056. doi: 10.1016/j.jpain.2014.07.005

Meulders, A., Harvie, D. S., Moseley, G. L., & Vlaeyen, J. W. (2015). Generalization of pain-related fear using a left-right hand judgment conditioning task. *Behavior Therapy*, *46*, 699–716. doi: 10.1016/j.beth.2015.02.004

Meulders, A., Jans, A., & Vlaeyen, J. W. (2015). Differences in pain-related fear acquisition and generalization: An experimental study comparing patients with fibromyalgia and healthy controls. *Pain*, *156*, 108–122. doi: 10.1016/j.pain.0000000000000016

Meulders, A., Vansteenwegen, D., & Vlaeyen, J. W. (2012). Women, but not men, report increasingly more pain during repeated (un)predictable painful electrocutaneous stimulation: Evidence for mediation by fear of pain. *Pain*, *153*, 1030–1041. doi: 10.1016/j.pain.2012.02.005

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *American Psychologist*, *61*, 10–26. doi: 10.1037/0003-066X.61.1.10

Moseley, G. L. (2003). Joining forces—Combining cognition-targeted motor control training with group or individual pain physiology education: A successful treatment for chronic low back pain. *Journal of Manual & Manipulative Therapy*, *11*, 88–94. doi: 10.1179/106698103790826383

Moseley, G. L. (2004). Evidence for a direct relationship between cognitive and physical change during an education intervention in people with chronic low back pain. *European Journal of Pain*, *8*, 39–45. doi: 10.1016/S1090-3801(03)00063-6

Moseley, G. L., & Flor, H. (2012). Targeting cortical representations in the treatment of chronic pain: A review. *Neurorehabilitation and Neural Repair*, *26*, 646–652. doi: 10.1177/1545968311433209

Moseley, G. L., & Vlaeyen, J. W. (2015). Beyond nociception: The imprecision hypothesis of chronic pain. *Pain*, *156*, 35–38. doi: 10.1016/j.pain.0000000000000014

Moseley, G. L., & Wiech, K. (2009). The effect of tactile discrimination training is enhanced when patients watch the reflected image of their unaffected limb during training. *Pain*, *144*, 314–319. doi: 10.1016/j.pain.2009.04.030

Moseley, G. L., Zalucki, N. M., & Wiech, K. (2008). Tactile discrimination, but not tactile stimulation alone, reduces chronic limb pain. *Pain*, *137*, 600–608. doi: 10.1016/j.pain.2007.10.021

Pavlov, I. P., & Gantt, W. (1928). *Lectures on conditioned reflexes: Twenty-five years of objective study of the higher nervous activity (behaviour) of animals*. New York, NY: International Publishers.

Perneger, T. V. (1998). What's wrong with Bonferroni adjustments. *British Medical Journal*, *316*, 1236. doi: 10.1136/bmj.316.7139.1236

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological Assessment*, *7*, 524–532. doi: 10.1037/1040-3590.7.4.524

Vlaeyen, J., & Linton, S. J. (2000). Fear-avoidance and its consequences in chronic musculoskeletal pain: A state of the art. *Pain*, *85*, 317–332. doi: 10.1016/S0304-3959(99)00242-0

Vlaeyen, J. W., de Jong, J., Geilen, M., Heuts, P. H., & van Breukelen, G. (2001). Graded exposure in vivo in the treatment of pain-related fear: A replicated single-case experimental design in four patients with chronic low back pain. *Behaviour Research and Therapy*, *39*, 151–166. doi: 10.1016/S0005-7967(99)00174-6

Vlaeyen, J. W., de Jong, J., Geilen, M., Heuts, P. H., & van Breukelen, G. (2002). The treatment of fear of movement/(re)injury in chronic low back pain: Further evidence on the effectiveness of exposure in vivo. *Clinical Journal of Pain*, *18*, 251–261. doi: 10.1097/00002508-200207000-00006

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., ... Aboyans, V. (2013). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*, 2163–2196. doi: 10.1016/S0140-6736(12)61729-2

Wand, B. M., Parkitny, L., O'Connell, N. E., Luomajoki, H., McAuley, J. H., Thacker, M., & Moseley, G. L. (2011). Cortical changes in chronic low back pain: Current state of the art and implications for clinical practice. *Manual Therapy*, *16*, 15–20. doi: 10.1016/j.math.2010.06.008

Zaman, J., Vlaeyen, J. W., Van Oudenhove, L., Wiech, K., & Van Diest, I. (2015). Associative fear learning and perceptual discrimination: A perceptual pathway in the development of chronic pain. *Neuroscience & Biobehavioral Reviews*, *51*, 118–125. doi: 10.1016/j.neubiorev.2015.01.009