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<i>Author contact at KU Leuven</i>	ann.meulders@ppw.kuleuven.be

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Differences in pain-related fear acquisition and generalization: An experimental study comparing
fibromyalgia patients and healthy controls

Ann Meulders^{1,2}, Anne Jans¹, & Johan W.S. Vlaeyen^{1,2,3}

¹ Research Group on Health Psychology, University of Leuven, Leuven, Belgium

² Center for Excellence on Generalization Research in Health and Psychopathology, University of
Leuven, Leuven, Belgium

³ Department of Clinical Psychological Science, Maastricht University, The Netherlands

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Correspondence concerning this article should be addressed to Ann Meulders, Ph.D, Department of
Psychology, University of Leuven, Tiensestraat 102, box 3726, 3000 Leuven, Belgium. E-
mail: ann.meulders@ppw.kuleuven.be, T: +32 (0)16 32 60 38, F: +32 (0)16 32 61 44.

Abstract

Anomalies in fear learning, such as the failure to inhibit fear to safe stimuli, lead to sustained anxiety, which in turn may augment pain. In the same vein, stimulus generalization is adaptive as it enables individuals to extrapolate the predictive value of one stimulus to similar stimuli. However, when fear spreads in an unbridled way to novel technically safe stimuli, stimulus generalization becomes maladaptive and may lead to dysfunctional avoidance behaviors and culminate in severe pain disability. In a voluntary movement conditioning paradigm, we compared the acquisition and generalization of pain-related fear in fibromyalgia patients (FM) and healthy controls (HC). During acquisition, participants received predictable pain in one context (i.e., one movement predicts pain, another does not), and unpredictable pain in another (i.e., pain never contingent upon movement). Fear generalization to novel movements (resembling the original painful or non-painful movement) was tested in both contexts. Results indicated that the FM group showed slower differential acquisition of pain-related fear in the predictable context, and more contextual pain-related fear in the unpredictable context. Fear of movement-related pain spreads selectively to novel movements similar to the original painful movement, and not to those resembling the non-painful movement in the HC, but non-differential fear generalization was observed in FM. As expected, in the unpredictable context, we also observed non-differential fear generalization; this effect was more pronounced in FM. Given the status of *overgeneralization* as a plausible transdiagnostic pathogenic marker, we believe this research might increase our knowledge about the pathogenesis of musculoskeletal widespread pain.

1. Introduction

Fibromyalgia (FM) syndrome is a chronic widespread pain condition characterized by high comorbidity with anxiety, fatigue, cognitive impairment and depression [54]. The relationship between fibromyalgia and anxiety is particularly firm[1] and evidence suggests that anxiety sensitivity often paves the way for the onset of chronic musculoskeletal pain[2]. Specifically, pain-related fear is considered to play a pivotal role in the origin and maintenance of chronic pain disability[14; 60; 61], a notion that is steadily receiving support[19; 31; 55; 56]. Associative learning mechanisms are shown to be crucially involved in the development of pain-related fear[41]. For instance, in a pain-related fear conditioning procedure, one neutral movement (conditioned stimulus, CS+) that is paired with pain (unconditioned stimulus, pain-US) starts to elicit fear/avoidance, whereas another movement (CS-) that is never paired with the pain-US does not. Fear learning has an important adaptive advantage – the ability to identify cues that signal threat (e.g. pain) can initiate appropriate defensive responses (e.g. avoidance) that protect us from harm, while identifying safety signals allows for goal-directed approach behaviors. Anomalies in fear learning, such as the failure to inhibit fear to safety signals (CS-) lead to sustained/generalized anxiety[3; 13; 22; 44] which in turn may augment pain[40; 48]. Moreover, a meta-analysis on fear conditioning studies[37] and recent experimental findings[18; 26; 32; 39] suggest that pathological anxiety is associated with impaired inhibition rather than disproportionate fear to danger signals (CS+).

Even adaptive learners face the challenge of how to deal with variations in the appearances of signaling stimuli. Stimulus generalization[20; 29] is an adaptive mechanism as it enables individuals to extrapolate the predictive value of one stimulus to similar stimuli and minimizes the necessity to learn everything anew. However, unbridled (over)generalization may lead to widespread fear, dysfunctional avoidance behaviors and culminate in severe pain disability[36; 38]. Using a voluntary joystick movement paradigm[41], we recently tested fear generalization towards

novel movements in two different contexts. In the predictable context, one arm movement (e.g. left) was followed by painful electrical stimulation and another was not (e.g. right). In the unpredictable context, the pain-US was never paired with the movements (e.g. upward/downward) but was presented during the context (intertrial interval). As expected, healthy individuals were more afraid of the movements that resembled the original CS+ than those resembling the CS- (i.e. differential fear generalization) in the predictable context. No such difference occurred in the unpredictable context, but a non-differential increase in fear was observed in this context [42].

In this study, we used the same approach to compare the acquisition and generalization of both cued and contextual pain-related fear in a chronic pain population (FM) with healthy controls (HC). Investigating (over)generalization –possibly a transdiagnostic marker of pathology– might further our understanding of the spreading of pain-related fear and pain in widespread musculoskeletal pain. We predicted 1) slower/less (adaptive) differential cued pain-related fear acquisition due to impaired safety learning, 2) more contextual pain-related fear during acquisition and generalization, 3) less differential (maladaptive) fear generalization, and 4) lower pain thresholds in FM than in HC.

2. Methods and Materials

2.1. Participants

This study used a convenience sample of 48 female participants including two age-matched diagnostic groups¹: 24 fibromyalgia patients (mean $\pm$ SD (range) age = 39 ± 13 (19–60) years), and 24 healthy controls (mean $\pm$ SD (range) age = 38 ± 13 (18–61) years). The most important inclusion criterion for the fibromyalgia group (FM) was to be diagnosed with fibromyalgia and experiencing some interference in their daily life because of this condition. All patients satisfied the American College of Rheumatology (ACR) new diagnostic criteria for fibromyalgia[65] based on the combined Widespread Pain Index (WPI; range 0-19) and Symptom Severity Score (SS; range 0-12) (see Table 1). The inclusion criterion for the healthy control group (HC) was to not have fibromyalgia. Exclusion criteria for both groups were: any other chronic pain conditions, diagnosed dyslexia or analphabetism, pregnancy, current or history of cardiovascular disease, chronic or acute respiratory disease (e.g., asthma, bronchitis), neurological diseases (e.g., epilepsy), uncorrected hearing problems, having pain at the dominant hand, wrist or arm that hinders to move a joystick painlessly, cardiac pacemaker or the presence of any other electronic medical devices, and the presence of any other severe medical conditions. An additional exclusion criterion only for the healthy control group was: any current or past psychiatric disorder including clinical depression and panic/anxiety disorder. Because of its high comorbidity with depression, other mood disorders and anxiety[33; 57; 64] this additional criterion was omitted in the fibromyalgia group. Fibromyalgia patients were recruited from pain clinics in the regions Flemish-Brabant and Limburg (Belgium) and through a call for participants on the website of the Flemish League for Fibromyalgia Patients. Following the procedure of Schneider et al.[50], patients were asked to bring a physically and mentally healthy friend of their own age (range) to the study. The experimental protocol was

¹ Note – we did not use absolute age-matched groups, but 5-year ranges to match the healthy controls to the fibromyalgia group. We do not think that the capacity to give verbal fear ratings or the fear-potentiated startle measures would significantly differ within the proposed age ranges.

approved by the Ethical Committee of the Faculty of Psychology and Educational Sciences of the University of Leuven (registration number: S-55100) and the Medical Ethical Committee of the University Hospital of the University of Leuven (registration number: ML9402). All participants signed the informed consent form, which explicitly stated that they were allowed to decline participation at any time during the experiment. As expected, FM patients had lower educational level, were more likely to be unemployed and were taking more medication than the healthy controls. More detailed demographic and clinical characteristics can be found in Table 1.

2.2. *Pre-experimental measures*

Prior to the data collection, all participants completed a battery of questionnaires using a web survey tool. The scores on these questionnaires can be found in Table 2. (1) Pain severity: The Chronic Pain Grade Scale (CPGS)[62] assesses pain intensity and interference with normal daily activities using 7 items (e.g., *How would you rate your pain at this moment?*). Answers on 6 of the 7 items range from 0 “no pain” to 10 “pain as bad as it could be”. The one remaining item requires filling in the number of days that pain has kept respondents from their typical daily activities in the last 6 months (range 0-180). Based on the pain intensity score, the disability points (based on the disability score and the days of disability) respondents are classified in four grades of chronic pain: Grade I, low disability–low intensity; Grade II, low disability –high intensity; Grade III, high disability–moderately limiting; and Grade IV, high disability–severely limiting. (2) Pain cognitions: the Pain Cognition List (PCL) [58] consists of 39 items divided into five subscales (Catastrophizing, Limitation, Optimism, Internal control and Trust). Each item presents a specific pain cognition statement (e.g., *“My thoughts are always concentrated on the pain”*) and the respondent is asked to indicate (dis)agreement on a 5-point Likert scale. Items are scored from 1 “totally disagree” to 5 “totally agree”, and a sum score is obtained per subscale (Catastrophizing: range 16-80; Limitation: range 7-35; Optimism: range 7-35; Internal control: range 5-25; Trust:

range 4-20). (3) Fear of movement: the Tampa Scale of Kinesiophobia (TSK)[43; 49] comprises 17 items intended to assess fear of movement and fear of (re)injury. Respondents are asked to indicate to what extent each of the statements (e.g., “*My body tells me that there is something seriously wrong with it*”) reflects a true description of the assumed association between movement and (re)injury on a four-point Likert scale, ranging from 1 “strongly disagree” to 4 “strongly agree” (total score range 17-68). (4) Pain disability: the Fibromyalgia Impact Questionnaire (FIQ)[10] assesses the impact of fibromyalgia on the respondent’s daily activities. The FIQ is composed of 10 items. The first item contains 11 questions (e.g., “*Can you independently do the dishes*”) related to physical functioning – each question is rated on a 4-point Likert type scale. Items 2 and 3 ask the respondent to mark the number of days they felt well and the number of days they were unable to work (including housework) because of pain symptoms. Items 4 through 10 are horizontal linear scales marked in 10 increments on which the respondent rates work difficulty, pain, fatigue, morning tiredness, stiffness, anxiety and depression. After normalization, the total score ranges from 0-100, with 0 indicating no impairment at all and 100 maximum impairment. (5) Affect: the trait version of the Positive and Negative Affect Schedule (PANAS)[16; 63] consists of 20 items divided into 2 subscales. Participants are asked to indicate to what extent, *in their normal daily life*, they experience the feelings defined by the 20 descriptors using a 5-point response scale ranging from “very little” to “a lot”. Ten items describe positive feelings and assess positive affectivity (PA; range 10-50) and 10 items describe negative feelings and assess negative affectivity (NA; range 10-50). (6) Depression and anxiety: the Hospital Anxiety Depression Scale (HADS)[52; 66] consists of 14 items divided into 2 subscales (Anxiety and Depression). Respondents are asked to indicate for each item (e.g., “*I still enjoy the things I used to enjoy*”) which answer reflects best how they felt during last week. Answers are scored from 0 to 3. The scores for the Depression subscale and the Anxiety subscale range from 0 to 21.

2.3. Stimulus material and experimental measures

The experiment was run on a Windows XP computer (Dell Optiplex 755) with 2 GB RAM and an Intel Core2 Duo processor at 2.33 GHz and an ATI Radeon 2400 graphics card with 256 MB of video RAM, using Affect 4.0[53]. We used four proprioceptive stimuli (i.e., moving a Logitech Attack 3 joystick in the horizontal plane (left/right) and in the vertical plane (upward/downward)) as CSs. The GSs were diagonal movements, (i.e., left-top, right-top, left-bottom, right-bottom). An electrocutaneous stimulus (2 ms duration) delivered by a commercial constant current stimulator (DS7A, Digitimer, Welwyn Garden City, England) served as the pain-US. This stimulation was administered through surface Sensormedics electrodes (8 mm) filled with K-Y gel that were attached to the wrist of the dominant hand. During the calibration procedure participants received a series of electrocutaneous stimuli of increasing intensity and were asked to indicate how intense/painful each stimulus was on a scale from 1 to 10 where ‘1’ means: “*you feel something but this is not painful, it is merely a sensation*”; ‘2’ means: “*this sensation starts to be painful, but it is still a very moderate pain*” up to ‘10’ which means: “*this is the worst pain you can imagine*”. A subjective stimulus intensity of ‘8’ which refers to a stimulus that is “*significantly painful and demanding some effort to tolerate*” was targeted. We used a standard protocol to increase the intensity level at each step of this calibration procedure. For all participants, the first electrocutaneous stimulus presentation had an intensity of 1mA, the next stimulus presentations respectively had an intensity of 2mA, 4mA, 6mA and 8mA, then the steps were increased from 2mA to 3mA increases between each stimulus presentation (until 20mA), finally the steps are again increased from 3mA to 4mA increases between each stimulus presentation, until the participant indicates that the stimulus is “*significantly painful and demanding some effort to tolerate*” which corresponds to an 8 on the 11-point intensity scale. The mean subjective stimulus intensity was 8.13 (SD = 0.61, range = 7–10) for the FM group, and 8.38 (SD = 0.71, range = 6–9) for the HC group. The mean physical stimulus intensity (in mA) was 26.46 (SD = 11.91, range = 6–48) for the FM group, and 37.46 (SD = 14.70, range = 14–68) for the HC group.

Conditioned pain-related fear was measured through self-reports as well as a psychophysiological measure of fear learning, that is, the eyeblink startle response. The eyeblink startle response is a component of the reflexive cross-species, full-body defensive response mobilization which is triggered by startle-evoking stimuli (e.g., acoustic startle probe) and can be measured by the tension in the muscles underneath the eye. *Startle modulation* refers to the potentiation of the startle reflex during fear states elicited by the anticipation of an aversive stimulus (e.g., an electrocutaneous stimulus). In the present setup, the startle probe was a 100 dBA burst of white noise with instantaneous rise time presented binaurally for 50 ms through headphones (Philips SHP2500). Eyeblink startle responses elicited by startle probes delivered *during the CS/GS movements* served as an index of cued pain-related fear. Eyeblink startle responses elicited by startle probes *during the intertrial interval (ITI)* served as an index of contextual pain-related fear[59].

2.4. Procedure

The procedure is largely the same as described by Meulders and Vlaeyen[42]. Important procedural adjustments were that 1) the original CS movements were also performed during generalization, with reinforced CS+ movements. This was done to prevent extinction, and to facilitate the transfer of the original acquisition task setting as much as possible to the generalization test, 2) we did not include latency measures as a proxy for avoidance behavior because we expected that FM would be overall slower for several reasons (e.g., medication, impaired working memory, attentional bias) which would make it difficult to interpret these data. The experiment was conducted during a 2-hour session and consisted of a preparation phase, a practice phase, a habituation phase, an acquisition phase, a transfer of acquisition phase, and a generalization phase. In a mixed design (see Table 3) both participants of the FM group and the HC group (between-subjects factor) received 1) predictable pain stimuli in one context and 2) unpredictable pain stimuli in another context (within-subjects factor). Half of the participants in

each group moved the joystick in the horizontal plane (left/right) in the predictable context, and in the vertical plane (upward/downward) in the unpredictable context. The other half of the participants in each group received the reverse combination. In the predictable context, one movement (CS_{p+}) was consistently followed by the pain-US and the other movement (CS_{p-}) was never followed by the pain-US. Note that the direction of joystick movement that served as the CS_{p+} and the CS_{p-} in the predictable context was counterbalanced across participants as well. In the unpredictable context however, the pain-US was never delivered contingent on either of the joystick movements (CS_{u1} and CS_{u2}) but was presented during the context (ITI; intertrial interval). During acquisition, participants freely choose on each trial in which direction they were going to move the joystick. During the transfer of acquisition, however, they could no longer choose the order of the movements themselves, but the movement direction was signaled. During generalization, the same signaling procedure was used to test the spreading of conditioned fear to novel diagonal movements (GSs).

2.4.1. Preparation phase

Upon arrival to the laboratory, participants were informed that the experiment involved the repeated presentation of electrocutaneous stimuli (pain-US) and short loud noises (acoustic startle probes). Participants were also told that they were free to decline participation at any time without any negative consequences. After providing informed consent, electrodes for eyeblink startle responses and the administration of the electrocutaneous stimulus were attached and the calibration procedure of the pain-US was initiated (see '2.3. Stimulus material and measures' section).

2.4.2. Practice phase

Before starting the practice phase, participants received extensive written instructions about the experimental task. In each block, participants were requested to move the joystick eight times as quickly and accurately as possible when prompted by a starting signal "+" (fixation cross presented

in the middle of the computer screen), in whatever order they freely chose. The position of *counter bars* on the computer screen indicated in which movement plane (horizontal vs. vertical) they were to move. The counter bars, each divided in four equal segments, always appeared on two sides of the computer screen (left/right or top/bottom). In a horizontal block, these bars were displayed on the left and right side of the computer screen, whereas in the vertical block, these bars appeared at the top and the bottom of the computer screen. Successful movements always resulted coloring one segment of the corresponding counter bar blue. That way, participants could instantly ascertain how many movements in each direction still were to be performed. In total, two blocks of eight trials were run: one block (4left/right) in the horizontal plane and one block (4upward/downward) in the vertical plane or vice versa. During the practice phase, no acoustic startle probes or pain-USs were presented and the experimenter gave online verbal feedback about the task performance.

2.4.3. *Startle habituation phase*

Because it is expected that the first responses to the startle probes are significantly larger than the latter ones, we inserted a startle probe habituation phase to correct for such possible confound in the data collection. This phase consisted of 12 trials, each lasting 24 s (with a variable ITI of on average 5 s (± 2 s)). During each trial one startle probe was presented either between the 1st and the 2nd second (three trials), at the 10th second (two trials), or between the 15th and the 17th second (three trials) after trial-onset. During this phase the participants wore headphones, and the lights in the experimental room were dimmed. Note that no pain-USs were delivered during this phase.

2.4.4. *Acquisition phase*

This phase was basically the same as the practice phase (see Fig. 1), but now 1) pain-USs and startle probes were presented, 2) six blocks (three predictable and three unpredictable) of eight trials were run instead of two blocks, and 3) instructions emphasized to pay close attention to the

starting signal “+” and to respond as fast and accurately as possible upon its presentation. Although a CS movement was of variable length depending on the participants’ movement speed, a trial typically included an ITI consisting of a pre-CS-interval of 3.5 s and a post-CS-interval of 8 s. The pain-US was presented within each CS_{p+} trial in the predictable context (100% contingency) and in half of the trials during ITI in the unpredictable context. In the predictable context, the US occurred immediately after the CS_{p+} movement. In the unpredictable context, the pain-US occurred between 1-3 s before the presentation of the starting signal “+”, or between 0.5-2.5 s after the CS movement. In each block with eight CS movements, four of the startle probes were presented during the CS movements (two during CS_{p+} or CS_{u1}, and two during CS_{p-} or CS_{u2}), and four during the ITI (two probe positions before the CS and two probe positions after the CS). To avoid that startle facilitation during ITI would be confounded by direct responses to presentations of the pain-US in the unpredictable context, startle probes were presented before the CS when the pain-US was presented after the CS, and after the CS when the pain-US was presented before the CS. Note that we did not inform the participants about the contingencies between the joystick movements (CSs) and the pain-US. After each conditioning block, the participants rated the cued pain-related fear elicited by each of the CS movements.

2.4.5. Transfer of acquisition

Transfer of acquisition trials were identical as those during the acquisition phase. Yet, CS movements were no longer voluntarily initiated but signaled. That is, 3000 ms after trial onset, a red asterisk “*” appeared for 500 ms at one of the CS movement directions (left/right in a horizontal block; top/bottom in a vertical block) indicating in which direction participants were requested to move. Before actually performing the signaled movement, participants rated their US-expectancy and cued pain-related fear. After completing the ratings, they waited for the “+” starting signal to start moving into the signaled direction. After successfully performing the signaled CS movement, a post-CS-ITI of 8 s followed (cfr. timing acquisition). During the transfer phase, two blocks (one

predictable and one unpredictable) of eight trials were run. Startle probes presentation followed the same timing schedule as during acquisition.

2.4.6. Generalization phase

The procedure of the generalization phase was mainly the same as the transfer of acquisition phase. The difference was that participants now had to perform four novel diagonal movements (GSs) which each have either a feature in common with the original CS_{p+} or the original CS_{p-}, namely left-top, right-top, left-bottom or right-bottom. Again, 3000 ms after trial onset, a red asterisk ‘*’ appeared for 500 ms in one of the corners of the screen to signal which movement had to be performed in randomized order after answering the US-expectancy and cued pain-related fear questions. After successfully performing a movement, a post-GS-ITI of 8 s followed (cfr. timing acquisition). On each trial a startle probe was delivered during the GS movement, original CS+ movement was still reinforced as during the previous phases, but none of the GSs was followed by the pain-US. During this phase, no ITI probes were delivered. The four GS movements were performed two times in each experimental context (predictable vs. unpredictable), the order was randomized across participants.

2.5. Manipulation checks and outcome variables

2.5.1. Manipulation checks

Contingency awareness. As a manipulation check, we assessed participants contingency awareness after the entire experiment using a retrospective US-expectancy rating. Participants indicated for both CS movements in each context how much they expected the painful stimulus to occur on an 11-point Likert scale (range 0-10) with labels ‘*not at all*’ to ‘*very much*’.

US-expectancy during transfer of acquisition. During the transfer of acquisition phase, participants indicated *before each movement* to what extent they expected the painful stimulus to occur when performing the “*signaled*” movements (CSs) on an 11-point Likert scale (range 0-10)

with labels ‘*not at all*’ to ‘*very much*’. That way, we could assess whether the contingencies learned during the acquisition phase using the *voluntary movement set-up* transfer to the *signaled movement set-up*.

2.5.2. *Self-reported fear of movement-related pain*

After each block, participants answered the following question: “*How afraid were you to perform the left/right/upward/downward movement?*” on an 11-point Likert scale ranging from 0 to 10 with anchors ‘*not fearful at all*’ to ‘*the worst fear you can imagine*’. Note that these questions applied to the previous block only (i.e., after a horizontal block ratings for the left/right movements and after a vertical block ratings to the upwards/downwards movements). During the transfer of acquisition and the generalization phases, participants rated *before each movement* how afraid they were to actually perform the “*signaled*” movements (CSs/GSs).

2.5.3. *US-expectancy during generalization*

During the generalization phase, participants rated *before each movement* to what extent they expected the painful stimulus to occur when performing the “*signaled*” movements (CSs/GSs) on an 11-point Likert scale (range 0-10) with labels ‘*not at all*’ to ‘*very much*’.

2.5.4. *Eyeblink startle modulation*

Orbicularis Oculi electromyographic activity (EMG) was recorded with three Ag/AgCl Sensormedics electrodes (4 mm) filled with electrolyte gel. After cleaning the skin with exfoliating peeling cream to reduce inter-electrode resistance, electrodes were placed on the left side of the face according to the site specifications proposed by Blumenthal et al. [2]. The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75–04). The recording bandwidth of the EMG signal was between 90 Hz and 1 kHz (± 3 dB). The signal was rectified online and smoothed by a Coulbourn multifunction integrator (LabLinc v76–23 A) with a time

constant of 20 ms. The EMG signal was digitized at 1000 Hz from 200 ms before the onset of the auditory startle probe until 1000 ms after probe onset.

2.6. Experimental setting

Participants were seated in an armchair (0.6 m screen distance) in a sound-attenuated and dimmed experimental room, adjacent to the experimenter's room. Further verbal communication was possible through an intercom system; the experimenter observed the participants and their physiological responses online by means of a closed-circuit TV installation and computer monitors.

2.7. Response definition and data analysis overview

2.7.1. Response definition of the startle modulation

Using PSPHA [5], a modular script-based program, we calculated the peak amplitudes defined as the maximum of the response curve within 21 – 175 ms after the startle probe onset. All startle waveforms were visually inspected off-line, and technical abnormalities and artifacts were eliminated using the PSPHA software. Every peak amplitude was scored by subtracting its baseline score (averaged EMG level between 1 and 20 ms after the probe onset). The raw scores were transformed to z-scores to account for inter-individual differences in physiological reactivity. In order to optimize the visualization of the startle data and avoid negative values on the Y-axis, T-scores –a linear transformation of the z-scores– were used in the figures. Averages were calculated for responding during CS/GS movements and ITI separately for the predictable and the unpredictable contexts. Participants who failed to reach an elevated peak amplitudes compared to baseline on more than 50% of probed trials were considered non-responders and were excluded from further analyses. A total of 6 healthy controls and 9 fibromyalgia patients from our sample were excluded due to the absence of reliable startle eyeblink responses, therefore the statistical analyses of the psychophysiological data were run on a total sample of 33 participants.

2.7.2 Data analysis overview

We carried out a series of repeated measures ANOVAs to examine the differences between fibromyalgia patients and healthy controls on the respective dependent measures in the unpredictable pain context and predictable pain context separately . Because we had clear a priori hypotheses, we further analyzed the data using planned comparisons. We applied Holm-Bonferroni **corrections** [28], instead of Bonferroni adjustments, to correct for multiple comparisons and reduce the probability of a type I error because the Bonferroni method is considered to be too conservative and inflates the probability of a type II error [46]. The effect size indication η_p^2 is reported for all omnibus ANOVA effects. Statistical analyses for all dependent measures were run with Statistica 12 software (StatSoft, Inc, Tulsa, Okla).

3. Results

3.1. US characteristics and questionnaires

As expected, FM patients selected an electrocutaneous stimulus of a lower intensity than the participants in the HC group ($t(46) = 2.85, p < .01$), however, the selected stimulus was not rated as less painful by the FM group than by the HC group ($t(46) = 1.31, p = .20$), (see Table 1). In addition, the groups significantly differed on all measures (see Table 2). In comparison with the HC group, the FM group had (1) higher pain intensity ($t(46) = 13.27, p < .0001$), greater pain disability ($t(46) = 12.89, p < .0001$), and more days of being disabled during the last 6 months ($t(46) = 5.03, p < .0001$), (CPCS), (2) higher scores on the Catastrophizing ($t(46) = 3.95, p < .001$), Limitation ($t(46) = 9.15, p < .0001$), and Internal control ($t(46) = -2.80, p < .01$) subscales, but lower scores on the Optimism ($t(46) = -3.57, p < .001$) and Trust ($t(46) = -2.09, p < .05$) subscales (PCL) (3) more fear of movement and (re)injury ($t(46) = 3.83, p < .001$), (TSK), (4) more impairment in their daily life activities due to the pain ($t(46) = 12.40, p < .0001$), (FIQ), (5) lower positive affect ($t(46) = -4.06, p < .001$) and higher negative affect ($t(46) = 3.37, p < .01$), (PANAS), (6) higher scores on both the depression ($t(46) = 4.97, p < .0001$) and anxiety ($t(46) = 4.94, p < .0001$) subscales (HADS).

3.2. Manipulation checks

3.2.1. Contingency awareness

Mean retrospective US-expectancy ratings in the predictable pain context and the unpredictable pain context were analyzed as manipulation checks using two separate 2×2 [Group (FM/HC) x Stimulus Type (CS+/CS-)]² repeated measures (RM) ANOVAs. In the predictable context, ratings for the CS+ movements were higher than for the CS- movements (main effect of Stimulus Type: $F(1, 46) = 9.79, p < .01, \eta_p^2 = .18$) and fibromyalgia patients gave higher US-expectancy ratings irrespective of Stimulus Type (main effect of Group: $F(1, 46) = 5.15, p < .05, \eta_p^2 = .10$). The Stimulus Type x Group interaction was not significant ($F < 1, \eta_p^2 = .0003$). In the unpredictable context, there was no significant main effect of Stimulus Type, ($F < 1, \eta_p^2 = .01$), and no significant Stimulus Type x Group interaction ($F < 1, \eta_p^2 = .003$). Fibromyalgia patients tended to give higher US-expectancy ratings for both unpredictable movements, but the main effect of Group just failed to reach significance ($F(1, 46) = 3.64, p = .06, \eta_p^2 = .07$). These results indicate, that at least at the end of the experiment, both groups were aware of the CS-US contingencies that were presented during the joystick task.

3.2.2. US-expectancy during transfer of acquisition

To check whether participants transferred their knowledge about the stimulus contingencies acquired in the voluntary to the signaled movement set-up, we collected US-expectancy ratings before each movement during the transfer of acquisition phase (see Fig. 2). We analyzed the mean online US-expectancy ratings during the predictable pain context and the unpredictable pain context using two separate $2 \times 2 \times 4$ [Group (FM/HC) x Stimulus Type (CS+/CS-) x Trial (T1-4)] RM ANOVAs. In the predictable context, this analysis yielded a significant main effect for Stimulus

² Note that throughout this paper, the notations CS+ and CS- used in the descriptions of the statistical analyses and the figures, respectively refer to the CS_{p+} and the CS_{p-} in the predictable context, and to the unreinforced CSs, (i.e., CS_{u1} and CS_{u2} in the unpredictable context). With respect to the GSs in the figures and analyses including the generalization data, for the sake of simplicity, GS_{p+u1} and GS_{p+u2} and, GS_{p-u1} and GS_{p-u2} are referred to respectively as CS+/GS+.

Type, $F(1, 46) = 28.46, p < .001, \eta_p^2 = .38$, as well as a Stimulus Type x Trial interaction, $F(3, 138) = 14.78, p < .001, \eta_p^2 = .24$. Although this two-way interaction was not modulated by Group (Group x Stimulus Type x Trial interaction, $F < 1, \eta_p^2 = .02$), we conducted planned comparisons to examine the transfer effects within each group. As expected, in the predictable context both groups gave higher US-expectancy ratings for the CS+ movement than for the CS- movement at the end of the transfer phase (T4), (FM: $F(1, 46) = 16.08, p < .05$; HC: $F(1, 46) = 18.33, p < .05$). Interestingly, however, whereas the healthy controls showed differential learning immediately from the first transfer trial (T1), (HC: $F(1, 46) = 6.33, p < .05$), the fibromyalgia patients did not, (FM: $F < 1$). The analysis on the US-expectancy ratings in the unpredictable context revealed a significant Stimulus Type x Group x Trial interaction, $F(3, 138) = 2.92, p < .05, \eta_p^2 = .06$. None of the groups showed differences in US-expectancies for both CS movements (FM: $F(1, 46) = 1.56, p = .22$; HC: $F(1, 46) = 1.64, p = .21$). Taken together, it seems that the fibromyalgia patients have more difficulties transferring the previously learned contingencies to a novel task context (i.e. signaled vs. voluntary movement set-up).

3.3. Outcome variables

3.3.1. Self-reported fear of movement-related pain

Acquisition effects. We conducted two separate $2 \times 2 \times 3$ [Group (FM/HC) x Stimulus Type (CS+/CS-) x Block (ACQ1-3)] RM ANOVA on the mean pain-related fear ratings for the CS movements after the three acquisition blocks in both the predictable and the unpredictable pain context (see Fig. 3). In the predictable context, there was a significant main effect of Stimulus Type, $F(1, 46) = 21.92, p < .001, \eta_p^2 = .32$, and a marginally significant main effect of Group, $F(1, 46) = 3.27, p = .08, \eta_p^2 = .07$, indicating that pain-related fear ratings were overall slightly higher in the patient group than in the healthy control group. Of crucial importance, there was a significant Stimulus Type x Block interaction, $F(2, 92) = 3.77, p < .05, \eta_p^2 = .08$. Although, this two-way

interaction was not modulated by Group, we continued to test our a priori hypotheses. Planned comparisons revealed that in the predictable context, both groups reported more pain-related fear at the end of the acquisition phase (ACQ3) in response to the CS+ movement than in response to the CS- movement, (FM: $F(1, 46) = 11.93, p < .01$; HC: $F(1, 46) = 7.31, p < .05$). Interestingly, this differential fear learning was already acquired in the first block (ACQ1) in the healthy control group, $F(1, 46) = 7.78, p < .05$, but not in the fibromyalgia patient group, $F(1, 46) = 1.20, p = .28$. Furthermore, pain-related fear in response to the painful (CS+) movement did not seem to differ for both groups across the acquisition phase, $F(1, 46) = 1.36, p = .25$, but pain-related fear in response to the non-painful (CS-) movement was elevated in the fibromyalgia group, $F(1, 46) = 6.36, p < .05$. The analysis in the unpredictable context yielded a borderline significant main effect of Group, $F(1, 46) = 2.99, p = .09, \eta_p^2 = .06$. As expected, no differences in pain-related fear were observed between both unreinforced CS movements in either of the groups (both F s < 1). Interestingly, however, in the unpredictable context, fibromyalgia tended to report more pain-related fear in response to both movements as compared with healthy controls in the beginning of the acquisition phase (ACQ1), $F(1, 46) = 5.24, p = .027$, after Holm-Bonferroni corrections this effect was no longer statistically significant, ($p > .0125$). At the end of acquisition (ACQ3) there was no difference in pain-related fear in response to unpredictable movements between both groups, $F(1, 46) = 1.41, p = .24$.

Transfer of acquisition effects. To check whether participants transferred the pain-related fear acquired in the voluntary to the signaled movement set-up, we collected fear ratings before each movement during the transfer of acquisition phase (see Fig. 4). We analyzed the mean pain-related fear ratings in both contexts separately using $2 \times 2 \times 4$ [Group (FM/HC) x Stimulus Type (CS+/CS-) x Trial (T1-4)] RM ANOVAs. In the predictable context, this analysis generated a significant main effect for Stimulus Type, $F(1, 46) = 14.08, p < .001, \eta_p^2 = .23$, as well as a significant Stimulus Type x Trial interaction, $F(3, 138) = 3.61, p < .05, \eta_p^2 = .07$. Although, this

two-way interaction was not modulated by Group, we continued to test our a priori hypotheses. As expected, in the predictable context both groups reported more pain-related fear in response to the CS+ movement than the CS- movement at the end of the transfer phase (T4), (FM: $F(1, 46) = 9.06$, $p < .05$; HC: $F(1, 46) = 6.83$, $p < .05$). Interestingly, however, whereas the healthy controls transferred the acquired differential fear learning immediately to the first transfer trial (T1), (HC: $F(1, 46) = 10.91$, $p < .05$), the fibromyalgia patients did not, (FM: $F < 1$). In line with the data pattern observed during acquisition, pain-related fear elicited by the painful (CS+) movement was similar in both groups across the transfer phase, $F < 1$, but pain-related fear elicited by the non-painful (CS-) movement tended to be higher in the fibromyalgia group, $F(1, 46) = 4.40$, $p = .041$, after Holm-Bonferroni corrections this effect was no longer statistically significant, ($p > .0125$). In the unpredictable context, the analyses showed a significant main effect of Group, $F(1, 46) = 4.15$, $p < .05$, $\eta_p^2 = .08$, as well as a significant Stimulus Type x Group x Block interaction, $F(3, 138) = 2.69$, $p < .05$, $\eta_p^2 = .06$. None of the groups showed differences in pain-related fear between both CS movements (FM: $F < 1$; HC: $F(1, 46) = 1.53$, $p = .22$). Consistent with the acquisition data, fibromyalgia patients again tended to report more pain-related fear for both movements than the healthy controls in the beginning of the transfer phase (T1), $F(1, 46) = 4.42$, $p < .05$, but after Holm-Bonferroni corrections this effect was no longer statistically significant, ($p > .0125$). No differences in pain-related fear for both unpredictable movements were observed at the end of the transfer phase (T4), $F(1, 46) = 2.72$, $p = .11$. Basically, it seems that the fibromyalgia patients have more difficulties transferring the differential fear learning to a novel task context (i.e. signaled vs. voluntary movement set-up) and as a consequence are generally more anxious.

Generalization effects. To examine generalization of pain-related fear to the novel diagonal GS movements in the predictable and the unpredictable pain context, we ran two 2×4 [Group (FM/HC) x Stimulus Type (CS+/GS+/GS-/CS-)] RM ANOVAs (see Fig. 5). From this analysis in the predictable context a significant main effect of Stimulus Type, $F(3, 138) = 12.68$, $p < .001$, $\eta_p^2 =$

.22, and a marginally significant main effect of Group emerged, $F(1, 46) = 3.37, p = .07, \eta_p^2 = .07$. Although, the Stimulus Type x Group, interaction failed to reach significance, $F(3, 138) = 1.73, p = .16, \eta_p^2 = .04$, we continued to test our a priori hypotheses. . In line with previous research, planned comparisons confirmed that in the healthy controls, GS+ movements (diagonal movements proprioceptively related to the original CS+) elicited higher fear of pain reports during the generalization test than the GS- movements (diagonal movements proprioceptively related to the original CS-) when tested in the predictable context, $F(1, 46) = 6.00, p < .05$. In contrast, this differential pain-related fear generalization effect was not observed in the fibromyalgia group, $F(1, 46) = 2.03, p = .16$. In the unpredictable context, this analysis showed a borderline significant main effect of Group, $F(1, 46) = 4.01, p = .05, \eta_p^2 = .08$, and no significant main effect of Stimulus Type, $F < 1, \eta_p^2 = .01$. The Stimulus Type x Group interaction also was not significant, $F(3, 138) = 1.41, p = .24, \eta_p^2 = .03$. Planned comparisons revealed that the same diagonal GS+ and GS- movements did not elicit differential fear of pain ratings in either of the groups, (both $F_s < 1$). Interestingly, the novel movements tested in the unpredictable context tended to produce more fear in the fibromyalgia group than in the healthy control group, $F(1, 46) = 4.53, p = .039$, but after Holm-Bonferroni corrections this effect was no longer statistically significant, ($p > .017$). Basically, it seems that in the predictable context, the fibromyalgia patients' pain-related fear spreads in a less stimulus-specific way than in the healthy controls, and fear is generally higher in response to all novel movements in the unpredictable context.

3.3.2. *US-expectancy during generalization*

We analyzed the mean US-expectancy ratings in both contexts during the generalization test using two separate 2×4 [Group (FM/HC) x Stimulus Type (CS+/GS+/GS-/CS-)] RM ANOVAs. This analysis in the predictable context revealed a significant main effect for Stimulus Type, $F(3, 138) = 16.78, p < .001, \eta_p^2 = .28$. Both the main effect of Group, and the Stimulus Type x Group

interaction, $F(3, 138) = 11.13, p < .001, \eta_p^2 = .19$, failed to reach significance. Notwithstanding the absence of the two-way interaction, we further tested our hypotheses using planned comparisons. US-expectancies for the GS+ movements were elevated compared with those for the GS- movements in both groups but to a lesser extent in the patient group (HC: $F(1, 46) = 13.38, p < .05$; FM: $F(1, 46) = 5.89, p < .05$), (see Fig. 6). In the unpredictable context, the analysis did not show any significant main effects for Group or Stimulus Type (both $F_s < 1$), as well as no significant two-way interaction, $F(3, 138) = 1.80, p = .15, \eta_p^2 = .02$. Planned comparisons revealed that the same GS movements did not generate any differences in US-expectancy ratings in either of the groups, both $F_s < 1$.

3.3.3. Eyeblink startle modulation

We analyzed the mean startle responses during acquisition and transfer of acquisition with a $2 \times 2 \times 3 \times 4$ [Group (FM/HC) x Context (Predictable/Unpredictable) x Stimulus Type (CS+/CS-/ITI) x Block (ACQ1-3, ACQ*³)] RM ANOVA (see Fig. 7). The analysis comparing psychophysiological fear responding elicited during the CSs and the ITI in both contexts revealed significant main effects for Context, $F(1, 31) = 13.99, p < .001, \eta_p^2 = .31$, and for Block, $F(3, 93) = 12.97, p < .001, \eta_p^2 = .29$, indicating habituation, that is, across blocks the startle responses declined gradually. Importantly, the Stimulus Type x Group interaction was significant, $F(2, 62) = 6.86, p < .001, \eta_p^2 = .18$, confirming that the startle responses elicited during the ITI and the respective CSs differed significantly in both the predictable and the unpredictable context. None of the interactions with Group was significant (all $p_s > .05$, all $\eta_p^2 < .12$). Planned comparisons further demonstrated that in both groups mean startle amplitudes during the CS+ movement were higher than during the CS- movement, $F(1, 31) = 6.10, p < .05$. In the unpredictable context, no such difference was observed, $F(1, 31) = 1.21, p = .28$. Interestingly, startle amplitudes during the ITIs in the

³ Because startle responses elicited by a single probe are not sufficiently reliable, means were calculated for the transfer phase and included as a fourth acquisition block (ACQ*) in the startle analysis.

unpredictable context were more elevated than in the predictable context, $F(1, 31) = 21.22$, $p < .001$, suggesting that more contextual pain-related fear emerged in the unpredictable context.

To examine generalization of psychophysiological fearful responding to the novel diagonal GS movements, we carried out a $2 \times 2 \times 4$ [Group (FM/HC) x Context (Predictable/Unpredictable) x Stimulus Type (CS+/GS+/GS-/CS-) RM ANOVA)] (see Fig. 8). The pattern of differential startle responding was similar to that of the verbal fear ratings, but the analysis failed to show a significant Context x Stimulus Type interaction or a significant three-way interaction, both $F_s < 1$, both $\eta_p^2 = .01$). Because we had clear a priori hypotheses, planned comparisons were further calculated. Startle amplitudes elicited during the GS+ movements tended to be elevated compared with those during the GS- movements in the predictable context for the healthy control group, $F(1, 31) = 4.85$, $p = .035$, but after Holm-Bonferroni corrections this effect was no longer statistically significant, ($p = .0125$). There was no difference between the startle responses during both GS movements in the fibromyalgia group, $F < 1$. No differential startle amplitudes were elicited by the same GS movements in the unpredictable context (both $F_s < 1$).

4. Discussion

We examined the differences in the acquisition of fear of movement-related pain in both a predictable and unpredictable context and whether FM and HC differ in the spreading of fear towards novel movements that are more or less resembling the original (non-)painful movements. We used a similar approach as Meulders and Vlaeyen[42] in their study in healthy controls, except for some methodological improvements. We hypothesized that as compared to the HC, the FM group would show 1) slower/less differential pain-related fear acquisition due to impaired safety learning, 2) more contextual pain-related fear during acquisition and generalization, 3) less differential fear generalization, and 4) lower pain thresholds. The results can be summarized as follows:

First, in the predictable context, we *successfully demonstrated the acquisition of pain-related fear* to the painful CS+ movement, but not to the non-painful CS- movement, in both groups. This effect was expressed by elevated fear reports, higher startle amplitudes, and higher US-expectancies to the CS+ than to the CS-. As expected, no such differential fear learning emerged in the unpredictable context. Interestingly, both groups did not acquire this differential fear learning at the same time: whereas HC showed this effect after only one training block, it took longer for the FM to pick up the CS-US contingencies. Further, it appears that the fear to the CS- was higher in the FM than in the HC group, indicating a lack of safety learning rather than excessive fear to the CS+.

Second, in both groups, we successfully established *contextual pain-related fear* –as indicated by higher ITI startles– in the unpredictable than in the predictable pain context. More importantly, our second hypothesis was also supported, that is, contextual pain-related fear was more substantial in the FM than in the HC group. More indirect evidence was found in the pain-related fear reports: both unpredictable movements, as well as the non-painful CS- movement yielded higher fear ratings in the FM than in the HC group. This might be explained by a spill-over effect from the threatening/unpredictable context (i.e. contextual fear) that is greater in the FM group. Generally, these results corroborate previous findings in anxiety disorders (e.g., PTSD, panic disorder) suggesting that anxiety patients exhibit elevated contextual fear and/or impaired safety learning[21; 23; 25; 32; 38; 39; 45].

Third, we replicated *differential fear generalization* to the novel diagonal movements in the predictable context in the HC. More specifically, the GS+ movements produced higher fear ratings, higher US-expectancy ratings, and marginally significant higher startle responses than the GS- movements. As predicted, the spreading of cued pain-related fear was not stimulus-specific in the FM group, that is, fear responding generalized equally to the movements resembling the original CS+ and CS- movements. This was evident in both fear measures. In the US-expectancy however,

there was minimal evidence for differential generalization of the acquired contingencies, suggesting that the major anomaly resides in the fear response system (e.g. elevated baseline startle) rather than on the basic associative learning level (dissociations between response systems have been reported earlier[4; 51]). As expected, in the unpredictable context, the same GS movements did not expose a differential generalization effect in the HC. Neither the fear reports, nor US-expectancy ratings, nor startle responses differed between the GS+ and the GS- movements. The same generalization pattern was observed in the FM group, but again fear elicited by the GS stimuli was generally higher in the unpredictable context compared to the HC group. Finally, FM patients chose a lower pain-US intensity than the HC, corroborating the observation that pain thresholds (pressure, heat) are reduced in FM patients[5; 34; 35; 47]

Some other interesting observations deserve further attention. First, we switched from a voluntary movement set-up to a signaled movement set-up; this signaled procedure is necessary to indicate the novel diagonal movements that have to be performed during the generalization test. In line with previous findings, participants in the HC transferred their CS-US knowledge easily from the acquisition to the transfer phase, whereas this was not the case in the FM group. The US-expectancy as well as the fear ratings assessed before the actual execution of the movements show that in the beginning of the transfer phase, patients do not show any differential conditioned responding. Again, this effect is largely driven by increased fear to the CS- movement. Furthermore, although the difference in contextual pain-related fear dissipated by the end of acquisition, during the transfer phase, elevated pain-related fear ratings to both movements reappeared in the FM but not in the HC group. Altogether, these data suggest that adaptive differential pain-related fear, and safety learning in particular, transfers poorly to novel contexts in the FM group. Even more maladaptive, is the fear generalization in the FM group, that is, fear seems to spread to all novel movements whether these are related to the original painful or non-painful movements. This *overgeneralization* is seemingly contradictory: on the one hand, FM

patients overgeneralize their fear to all novel movements, but once the adaptive differential fear learning took place, it does not seem to generalize well to new contexts (i.e. *overdiscrimination*). Especially, inhibitory learning to the CS- movement seems fragile and context-dependent. This observation can be understood in terms of the well-known dissociation between first and second learned associations: acquisition learning (first learned association) normally generalizes easily across contexts, whereas extinction learning (second learned association that *inhibits* the behavioral expression of the first learned association) does not, but is more context-dependent[6-9]. By analogy, safety learning might be more vulnerable during context switches, especially in individuals characterized by fragile safety learning.

Second, based on the retrospective US-expectancy ratings FM patients acquired the CS-US contingencies, although they fail to inhibit their psychophysiological and self-reported fear to the non-painful movements during the task. These results contradict the findings of Jenewein et al.[30] demonstrating that only 50% of the FM were contingency aware as compared to 100% and 86% of the HC and the osteoarthritis group, respectively. This might be associated with the less clear-cut contingencies in their study. That is, a triangle (CS-) was always followed by a low temperature heat stimulus and a square (CS+) was either followed by a high or low temperature heat stimulus. Because of this partial reinforcement scheme, FM might have had more difficulties to discriminate accurately between cues that were sometimes or always followed by the low temperature.

Some limitations should be addressed as well. *First*, we excluded 15 non-responders from the startle analyses; general interactions might have failed to reach statistical significance due to reduced statistical power. The relatively high number of non-responders might be related to the inclusion of older adults who typically have weaker startle responses[17]. *Second*, emotions also fade when getting older (i.e. blunted affect), which is reflected in the lower fear measures compared with healthy student samples. Alternatively, this might be explained by social desirability in students or that patients' persistent real-life pain is more extreme than the experimental pain. *Third*,

this study cannot draw any conclusion regarding the causal relationship between poor acquisition and overgeneralization of pain-related fear and the development of FM, longitudinal studies are needed to disentangle the causal status of fear learning deficits in the development of FM. Nevertheless, we are convinced that this learning deficit might be involved in the maintenance of FM, because when potential danger/harm is not successfully identified, it boils down to sustained anxiety, further spreading of fear and proliferation of avoidance/defensive behaviors. *Fourth*, the groups also differed with respect to medication use[11; 15; 27] and comorbidity with anxiety[32; 38; 39] and depression thus we cannot exclude the possibility that this might have contributed to the observed differences in fear learning/expression. Indeed, anxiolytics might affect the expression of context conditioning[24], opioids have shown to impair fear learning[15], and antidepressants may enhance cued fear conditioning[12; 27]. However, given the possible opposite effects of the different drugs used in our patient group, it is rather unlikely that the medication use explains all the observed variance between FM and HC. *Fifth*, although some of the omnibus ANOVA interactions were not significant –probably due to a lack of statistical power– we continued testing our a priori hypotheses. Bonferroni corrections for multiple testing have been criticized for being too conservative, for increasing the probability of Type II errors and undermining the statistical power[46], therefore we used Holm-Bonferroni corrections.

Despite these limitations, the data seem to support the idea that increased anxiety levels in FM patients likely are associated with deficient safety learning, which in turn enhances (over)generalization of pain-related fear and the possibly maintenance of widespread pain. Future prospective studies with a longitudinal design are needed to further test this intriguing possibility.

To conclude, this is the first study demonstrating slower acquisition due to impaired safety learning and overgeneralization of pain-related fear in FM compared with HC. We believe that investigating (over)generalization –possibly a transdiagnostic marker of pathology– might help shed light on the pathogenesis of musculoskeletal widespread pain and the spreading of anxiety and pain.

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6. Financial disclosures

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Figure Captions

Figure 1. Schematic overview of the experimental task and trial timing during the acquisition phase for both the predictable context (A) and the unpredictable context (B) and during the generalization phase for both predictable context (C) and the unpredictable context (D).

Figure 2. Mean US-expectancy ratings ($\pm$ SE's) for the CS movements in the predictable and the unpredictable context assessed *before* each trial during the transfer of acquisition phase (T1-4).

Figure 3. Mean self-reported pain-related fear ($\pm$ SE's) in response to the CS movements in the predictable and the unpredictable context assessed *after* each block during acquisition (ACQ1-3).

Figure 4. Mean self-reported pain-related fear ($\pm$ SE's) in response to the CS movements in the predictable and the unpredictable context assessed *before* each trial during the transfer of acquisition phase (T1-4).

Figure 5. Mean self-reported pain-related fear ($\pm$ SE's) in response to the original CS movements (CS+/CS-) and the novel diagonal GS movements (GS+/GS-) in the predictable and the unpredictable context assessed *before* each trial during the generalization phase.

Figure 6. Mean US-expectancy ratings ($\pm$ SE's) for the original CS movements (CS+/CS-) and the novel diagonal GS movements (GS+/GS-) in the predictable and the unpredictable context assessed *before* each trial during the generalization phase.

Figure 7. Mean eyeblink startle amplitudes ($\pm$ SE's) during the CS movements and the ITI in the predictable and the unpredictable contexts during the acquisition. Note – that for graphic purposes T-scores were used.

Figure 8. Mean eyeblink startle amplitudes ($\pm$ SE's) during the original CS movements (CS+/CS-) and the novel diagonal GS movements (GS+/GS-) in the predictable and the unpredictable contexts during the generalization phase. Note – that for graphic purposes T-scores were used.

Figure 1

A. PREDICTABLE CONTEXT

B. UNPREDICTABLE CONTEXT

C. PREDICTABLE CONTEXT

D. UNPREDICTABLE CONTEXT

Note – the white “+” serves as the starting signal to initiate the voluntary movements during the acquisition phase (panel A and B). During the transfer of acquisition phase and the generalization phase (panel C and D), the order of the movements is no longer chosen freely, a red “*” indicates which CS/GS (diagonal) movement ought to be performed. Blue colored segments of the counter bars represent the number of performed movements and white colored segments of the counter bars indicate the movements that still ought to be performed. The white arrow represents the CS movement and the drawing of a lightning bolt represents the pain-US presentation. CS_{p+} and CS_{p-} respectively refer to the movement that is followed by the pain-US (i.e. left) and the movement that is never followed by the pain-US (i.e. right) in the predictable context, whereas CS_{u1} and CS_{u2} both refer to movements that are never followed by the pain-US in the unpredictable condition (i.e. upwards and downwards). GS movements are novel diagonal movements that have either one feature in common with the original CS_{p+} [GS_{p+u1} and GS_{p+u2}] or the original CS_{p-} [GS_{p-u1} and GS_{p-u2}]. During acquisition and transfer of acquisition, one startle probe is presented on each trial, in 50% of the trials during the ITI and on 50% of the trials during the CS movement. In the unpredictable context, ITI probes are presented before the movement (PRE-CS-ITI) if the unpredictable pain-US is delivered after the CS movement (POST-CS-ITI), and the ITI probes are presented after the CS movement (POST-CS-ITI) if the unpredictable pain-US is presented before the CS movement (PRE-CS-ITI). During the generalization phase, only CS/GS startle probes were presented.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Antidepressants	50%	0%
Anxiolytics	17%	0%
Analgesics	92%	8%
Other	67%	0%
Pain treatment (yes – no)	100%	29%

Note – WPI = Widespread Pain index: the higher the score, the more pain complaints on different sites of the body during the past week; SS = Symptom Severity Score: 0 = no symptoms, 12 = very much pain symptoms. Other medication includes: sleep medication, muscle relaxants, hormones, anti-hypertension, antiarrhythmic, proton pump inhibitor, leukotriene receptor antagonist and prevention for migraine medication. ** $p < .01$; *** $p < .001$.

Table 2. *Questionnaire scores for the fibromyalgia group and healthy control group separately.*

Total sample $N = 48$	Fibromyalgia group ($n = 24$)		Healthy control group ($n = 24$)			
	Mean	<i>SD</i>	Mean	<i>SD</i>	<i>t</i>	df
CPGS – pain intensity***	70.00	11.38	19.44	14.80	13.27	46
CPGS – pain disability***	60.97	19.72	5.69	7.26	12.89	46
CPGS – #days disability***	76.67	73.52	1.08	2.55	5.03	46
PCL – catastrophizing***	47.17	13.32	33.54	10.42	3.95	46
PCL – limitation***	28.00	3.60	17.42	4.37	9.15	46
PCL – optimism***	22.96	5.77	28.29	4.50	-3.57	46
PCL – internal control**	17.00	3.53	19.63	2.95	-2.80	46
PCL – trust*	14.00	2.25	15.54	2.83	-2.09	46
TSK – total score***	38.88	6.70	31.46	6.71	3.83	46
FIQ – total score***	61.50	15.34	16.53	8.95	12.40	46
PANAS – positive affect***	30.25	7.81	37.96	5.03	-4.06	46
PANAS – negative affect**	25.54	8.63	18.50	5.53	3.37	46
HADS – anxiety***	9.75	4.90	4.29	2.29	4.94	46
HADS – depression***	6.92	4.37	1.83	2.44	4.97	46

Note – CPGS = Chronic Pain Grade Scale: pain intensity (item 1-3), pain disability (item 4-6) and days of disability (item 7); PCL = Pain Cognition List: subscales are calculated for catastrophizing, limitation, optimism, internal control and trust; TSK = total score on the Tampa Scale of Kinesiophobia; FIQ = total score on the Fibromyalgia Impact Questionnaire; PANAS = Positive and Negative Affect Schedule: subscales are calculated for positive affect and negative affect. HADS = Hospital Anxiety Depression Scale: subscales are calculated for anxiety and depression. * $p < .05$, ** $p < .01$, *** $p < .001$. Based on the CPGS scales, 2% (1/24) of the fibromyalgia patients was classified as Grade I (low disability–low intensity), 8% (2/24) as Grade II (low disability –high intensity), 38% (9/24) as Grade III (high disability–moderately limiting) and 50% (12/24) as Grade IV (high disability–severely limiting).

Table 3

Summary of the experimental design

Context	Practice	Habituation	Acquisition	Transfer of acquisition	Generalization
Predictable	4xCS _p ⁺ only 4xCS _p ⁻	6 probes	12xCS _p ⁺ 12xCS _p ⁻	4xCS _p ⁺ 4xCS _p ⁻	4xCS _p ⁺ 4xCS _p ⁻ 2xGS _{p u1} ⁺ 2xGS _{p u1} ⁻ 2xGS _{p u2} ⁺ 2xGS _{p u2} ⁻
Unpredictable	4xCS _{u1} 4xCS _{u2}	6 probes	12xCS _{u1} 12xCS _{u2} 12xUS (during ITI)	4xCS _{u1} 4xCS _{u2} 4xUS (ITI)	4xCS _{u1} 4xCS _{u2} 2xGS _{p u1} ⁺ 2xGS _{p u1} ⁻ 2xGS _{p u2} ⁺ 2xGS _{p u2} ⁻

CS = conditioned stimulus; GS = generalization stimulus; US = unconditioned stimulus. CS_p⁺ = movement in predictable condition that was consistently followed by the painful stimulus (US); CS_p⁻ = movement in predictable condition that was never followed by the painful stimulus (US); CS_{u1} and CS_{u2} = movements in unpredictable context that were never followed by the painful stimulus (US). GSs = novel diagonal movements which share proprioceptive characteristics with the original CS_p⁺ [GS_{p u1}⁺ and GS_{p u2}⁺] or the original CS_p⁻ [GS_{p u1}⁻ and GS_{p u2}⁻]. For both experimental groups (FM and HC) the predictable and unpredictable pain conditions were run within-subjects in blocks of eight movements in a semi-randomized order (no more than two consecutive blocks could be in the same context). GS movements are never reinforced. The suffix “only” is used to indicate non-reinforcement of the CS_p⁺ movement (i.e. during the practice phase).