Extinction of fear generalization: a comparison between fibromyalgia patients and healthy controls

Ann Meulders[1,2], Michel Meulders[3,4], Iris Stouten[1], Jozef De Bie[5,6] & Johan W.S. Vlaeyen[1,2,7]

[1] Research Group Health Psychology, KU Leuven University, Leuven, Belgium

[2]Center for Excellence on Generalization Research in Health and Psychopathology, KU Leuven University, Leuven, Belgium

[3]Center for Information Management, Modeling and Simulation, KU Leuven University, Belgium

[4]Research Group on Quantitative Psychology and Individual Differences, KU Leuven University, Leuven, Belgium

[5]Multidisciplinary Pain Center, Ziekenhuis Oost-Limburg, Genk, Belgium

[6]Centre for Translational Psychological Research (TRACE), Ziekenhuis Oost-Limburg & KU Leuven, University Belgium

[7]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

Fear learning deficiencies might contribute to the development and maintenance of chronic pain disability. Fear is often not restricted to movements (conditioned stimulus=CS+) originally associated with pain (unconditioned stimulus=US), but expands to similar movements (generalization stimuli=GSs). This spreading of fear becomes dysfunctional when *overgeneralization* to safe stimuli occurs. More importantly, persistence of pain-related fear to GSs despite corrective feedback might even be more debilitating and maintain long-term chronic pain disability. Yet, research on this topic is lacking.

Using a voluntary joystick movement paradigm, we examined (extinction of) pain-related fear generalization in fibromyalgia patients (FM) and healthy controls (HC). During acquisition, one movement (CS+) predicted pain; another did not (CS-). We tested (extinction of) fear generalization to five GSs varying in similarity with the CS+ and CS-. Results revealed flatter pain-expectancy generalization gradients in FM than HC due to elevated responses to GSs more similar to the CS-; the fear generalization gradients did not differ. Although, pain-related fear and expectancy to the GSs decreased during extinction, responding to the GSs remained higher for FM than HC, suggesting that extinction of generalization is impaired in chronic pain patients. Persistence of excessive protective responses may contribute to maintaining long-term chronic pain disability.

Perspective: Pain-related fear and expectancy to movements –varying in similarity with the original painful and non-painful movement– decrease during extinction in healthy controls and fibromyalgia patients. Yet, conditioned responses remain elevated in patients despite corrective feedback, indicating impaired extinction of generalization. Persistent excessive protective responses may contribute to preserving pain disability.

1. Introduction

Accumulating evidence indicates that pain-related fear plays a fundamental role in the transition from acute to chronic disabling pain[59]. Fear can be defined as an (phasic) emotional response to the anticipation or imminence of threat, harm, or in the specific case of pain-related fear, pain. It is commonly accepted that fear can at least be expressed in three response systems[24]: (1) verbal responding (both cognition and affect), (2) psychophysiological responding (changes in autonomic responding and reflex modulation), and (3) behavioral changes (such as avoidance or removal from the source of threat). It has been shown repeatedly that pain-related fear can be acquired via associative learning[31-38, 52]. An initially neutral movement (conditioned stimulus=CS+) that is associated with pain (unconditioned stimulus=pain-US) starts to signal danger and evokes protective behavior (conditioned responses=CR), while the CS- (control stimulus never paired with the pain-US) does not trigger such responses. Fear learning is an adaptive mechanism, because the ability to identify cues signaling threat and safety enables protective action against potential bodily harm. In the clinic, however, spreading of fear and avoidance is observed beyond movements/activities that were associated with pain during the original pain episode. For example, when someone experiences a shooting back pain while lifting a box, it is possible (s)he will not only expect to feel pain and be fearful to lift this specific box, but also when lifting other objects (e.g. a baby, a shopping bag). One possible mechanism accounting for this spreading of fear is *stimulus generalization*[14, 19, 22]. Stimulus generalization is also adaptive as it enables individuals to extrapolate the predictive value of one stimulus to novel, similar stimuli without actually having to experience them. From an associative learning perspective this implies that CRs may extend to a range of novel stimuli resembling the original CS+, with more similar GSs evoking stronger CRs. Yet, together with reducing the risk of missing positive threat alarms, which may contribute to avoiding harm in a swiftly changing environment, generalization bears an increased risk to respond to false threat alarms. In particular, when fear spreads in an unbridled way, stimulus generalization becomes maladaptive and may lead to dysfunctional protective behaviors and culminate in severe pain disability.

In a previous study, we demonstrated that fear indeed spreads selectively towards novel movements resembling the original painful movement in healthy pain-free controls (HC), but fear generalized in a non-differential way in fibromyalgia patients (FM)[31]. In another study using a scenario contingency learning task with the verbal labels 'pain' and 'no pain' as outcomes and pictures of hand postures as cues, we found that chronic hand pain patients overgeneralized pain-outcome expectancy to novel cues that were more similar to the original 'safe' cue as compared to HC[30]. We argued that *excessive generalization* might be involved in the etiology of chronic pain disability by spreading of undesired protective behaviors. Moreover, persistence of pain-related fear and expectancy to technically safe, unreinforced GSs despite corrective feedback might even be more debilitating and maintain chronic pain disability in the long run, however to our knowledge this has never been tested. Therefore, this study aimed to test pain-related fear generalization and its extinction in FM and HC using a voluntary joystick movement conditioning task. We hypothesized that FM would show 1) flatter generalization gradients than HC due to higher responses to the GSs that resemble the original CS- more, 2) impaired extinction of unreinforced GSs, whereas generalized pain-related fear will subside quickly in HC.

2. Methods and Materials

2.1. Participants

This study used a convenience sample of 60 participants including two age-matched diagnostic groups: 30 fibromyalgia patients (29 females, mean ± SD (range) age = 41 ± 11 (22–59) years), and 30 healthy controls (mean ± SD (range) age = 41 ± 12 (21–60) years). In line with Meulders et al.[31], we chose not to use absolute age-matched groups, but used 5-year ranges to match the healthy controls (HC) to the fibromyalgia (FM) group. The most important inclusion criterion for the FM group 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[58] 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 HC group 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 HC 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[23, 48, 55] this additional criterion was omitted in the FM group. Participants were recruited via social media and from pain clinics in the Limburg region (Belgium). The study protocol was approved by the Social and Societal Ethics Committee of the KU Leuven (registration number: S-56226), the Medical Ethical Committee of Ziekenhuis Oost-Limburg (ZOL), and the Medical Ethical Committee of the University Hospital of KU Leuven (ML10116). All participants signed the informed consent form, which explicitly stated that they were allowed to decline participation at any time during the experiment. To compensate for their time and effort, FM patients received the book '*Mastering your Pain*' [de Pijn de Baas] by Frits Winter[57], HC received a box of Belgian chocolates; both remunerations had an approximate value of €15. 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.Stimulus material and apparatus

The experiment was run on a Windows 7 computer (Dell OptiPlex 7010) with 4096MB RAM and an Intel Core i5-3570 CPU processor at 3.40 GHz and an AMD Radeon HD 7570 graphics card with 2542MB of video RAM. Experimental stimuli were presented on a 19-inch computer screen and were controlled with the free experimental software package Affect 4.0[47]. The data were stored using a National Instruments data acquisition card (National Instruments Corporation, Austin, Texas). The

conditioned stimuli (CSs) and the generalization stimuli (GSs) were seven, equally spaced movement quadrants, which are part of a semi-circle (see Figure 1). These proprioceptive stimuli consisted of moving a Paccus Hawk joystick (Paccus Interfaces BV, Almere, The Netherlands) with the dominant hand within one of the seven movement quadrants. Movements in quadrant 1 (i.e. 90° to the left) and in quadrant 7 (i.e. 90° to the right) served as CSs, and movements in the intermediate (2-6) quadrants, served as GSs. During acquisition, one movement direction (CS+) was followed by the pain-US (75% of the trials), while the other movement direction was never followed by the pain-US (CS-); which movement quadrant (1 or 7) served as CS+ or CS- was counterbalanced across participants. The pain-US was a painful electrocutaneous stimulus (2 ms duration), generated by a commercial constant current stimulator (DS7A, Digitimer) and administered through surface Sensor Medics electrodes (8mm) filled with K-Y gel that were attached to the wrist of the dominant hand. Before the experiment started, participants went through a calibration procedure: they received a series of electrocutaneous stimuli of increasing intensity and were asked to indicate how intense 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. The mean self-reported stimulus intensity was 7.87 (SD = 0.35, range = 7–8) for the FM group, and 7.98 (SD = 0.18, range = 7–8) for the HC group. The mean physical stimulus intensity (in mA) was 18.73 (SD = 8.53, range = 8–48) for the FM group, and 22.90 (SD = 12.18, range = 11–60) for the HC group.

Conditioned pain-related fear was assessed through self-reports as well as a psychophysiological index 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.

2.3. 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.

2.4. Procedure

The experiment was conducted during a 2-hour session and comprised six experimental phases: a preparation phase, a practice phase, a habituation phase, an acquisition phase, a transfer-of-acquisition phase, and a generalization phase. The procedure is largely based on Meulders et al. [33]. In a mixed design (see Table 2) participants in both groups (between-subjects factor: FM *vs*. HC) received a pain-US after moving the joystick to the CS+ direction on 75% of the trials, but never received a pain-US when moving the joystick to the CS- direction (within-subjects factor: CS+ *vs*. CS-). Note that the movement direction that served as the CS+ and CS- was counterbalanced across participants. During acquisition, participants freely chose 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 intermediate movement directions (GSs).

2.4. 1. Preparation phase

Upon arrival to the laboratory, participants were informed that pain-USs and short loud noises (acoustic startle probes) would be administered during the experiment. Participants were also told that they were free to decline participation at any time without any consequences. Subsequently, written informed consent was obtained. Before selecting the intensity of the painful stimulus following the calibration procedure, electrodes for the eyeblink startle responses and the electrocutaneous stimulus were attached (see *'2.2. Stimulus material and apparatus'* section).

2.4.2. Practice

Figure 1 provides an illustrative overview of the design and the voluntary joystick movement task. Before starting the practice phase, participants received detailed instructions about the experimental task. At the beginning of each trial the cursor representing the joystick needed to be positioned in the middle of the screen, so that the joystick was standing upright and centered. When prompted by a starting signal "+" (fixation cross presented in the middle of the computer screen), participants moved the joystick as quickly and accurately as possible, in whatever order they freely chose. They were requested to move the joystick towards the *counter bars*, each divided in four equal segments, positioned at the end of the two equally spaced movement quadrants (1 and 7). Successful movements always resulted in 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. During this phase, visual feedback about the position of the joystick (i.e. visualized by the cursor) and the performance of the movements was provided on the computer screen. The movement quadrants were delineated with white borders. Whenever the cursor (representing the joystick position) entered a quadrant, this area turned green. When the cursor wrongly left the movement quadrant, this area turned red. During the practice phase participants completed 16 valid movements, that is, two blocks of eight trials (4left/4right) in total. No pain-USs or startle probes were presented during this phase, but the experimenter provided verbal feedback about the performance of the joystick movements.

2.4.3. Startle habituation

Because the first responses to the startle probes are usually relatively large, we included a phase to habituate to the probes, to correct for such possible confounds during the data collection. This habituation phase consisted of 8 trials, each lasting 12s, with an intertrial interval (ITI) of 2s. During each trial one startle probe (100 dBA burst of white noise) was delivered between the 8th and the 12th second after trial onset. Participants wore headphones, and the lights in the experimental room were dimmed. No pain-USs were delivered during this phase.

2.4.4. Acquisition

This phase was basically the same as the practice phase (see Figure 1), but now 1) no verbal feedback was administered about the task performance, 2) pain-USs and startle probes were presented, 3) the movement quadrants were not delineated with white borders and they did not turn green/red when the cursor entered/left the corresponding movement quadrant; the cursor was no longer visible for the participant, 4) instructions emphasized to pay close attention to the starting signal "+" and to respond as fast and accurately as possible upon its presentation.

The acquisition phase consisted of three blocks of 8 trials. Each block contained 4 trials to the left and 4 trials to the right. 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 s and a post-CS-interval of 8 s. The pain-US was presented on 75% of the CS+ trials, but never on CS- trials. In each block of eight CS movements, four of the startle probes were presented during the CS movements, and four during the ITI (between 3000 – 6000 ms after the movement was executed). Note that we did not inform the participants about the contingencies between the joystick movements (CSs) and the pain-US. After each conditioning block, participants rated the 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, with the exception that participants could no longer freely choose in which order they performed the CS

movements. More specifically, 3000 ms after trial onset, a green border was presented around the counter bars of one of the movement quadrants to indicate in which direction participants were to move. Before actually performing the movement, participants rated to what extent they expected to receive a pain-US after the to-be-performed movement, and to what extent they were afraid to perform that movement. After answering these questions, participants waited for the starting signal to appear and started moving into the signaled direction. After successfully performing the signaled CS movement, a post-CS-ITI of 8 s followed. During the transfer phase, one block of eight trials (4left/4right) was run. The reinforcement scheme and timing scheme of the presentation of the startle probes remained the same as during the acquisition phase.

2.4.6.Generalization

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 five novel movements (GSs) into intermediate movement quadrants (2-6) between the CS+ and the CS-. Movements to quadrant 1 or 7 still served as CS+ and remained reinforced at the same rate; the CS- and GSs, however, were never followed by the pain-US. The generalization phase consisted of 28 trials, in which participants performed 4 trials of each movement (i.e., quadrants 1-7). One block consists of 7 movements (one in each movement quadrant); the order of the movements was randomized across participants. As before, after a 3s pre-CS/GS ITI, the movement direction was signaled by a green border around the counter bar of the corresponding movement quadrant and participants rated their pain-US expectancy and pain-related fear. Next, the fixation cross appeared and participants moved the joystick into the signaled direction. After successfully performing the movement, again a post-CS/GS ITI of 8 s followed. On each trial a startle probe was delivered during the GS/CS movement; no ITI probes were delivered.

2.5. Measures

2.5.1. Manipulation checks

Affective valence, arousal and control. After the practice, acquisition and generalization phase, participants completed three Self-Assessment Manikin (SAM) scales[4] measuring affective valence, arousal and the control they experienced when performing the CS movements. The SAM scales each consisted of 5 different pictographs of humanlike figures – manikins. These manikins differ in emotional expressions ranging respectively from "happy" to "unhappy", "very aroused" to "calm", "no control" to "a lot of control". Participants selected the manikin that matched best how they felt when performing the respective CS movements. Responses were scored from 1 to 5 (happy/very aroused/no control– sad/calm/a lot of control).

2.5.2. Main outcome variables

Self-reported fear of movement-related pain. After each block, participants answered the following question: "*How afraid were you to perform the left/right movement?*" on an 11-point Likert scale ranging from 0 to 10 with anchors '*not fearful at all'* to '*extremely fearful'*. 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).

Pain-US expectancy during transfer-of-acquisition and generalization. During the transfer-of-acquisition and generalization phases, 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'*.

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.

Pain-US intensity and unpleasantness. After each block, participants indicated the unpleasantness and the intensity of the painful stimulus on an 11-point Likert scale (range 0-10) with labels "*not unpleasant at all*" and "*very unpleasant*", and "*not painful at all*" and "*very painful*".

2.5.3 Post-experimental questionnaires

After the data collection, all participants completed a battery of questionnaires to map out possible psychological differences between the FM group and the HC group using a web survey tool. The scores on these questionnaires can be found in Table 3. (1) Pain severity: The Chronic Pain Grade Scale (CPGS)[53] 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)[49] 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] 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)[5] 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)[12, 54] 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)[46, 60] 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. (7) Fear of pain: The Fear of Pain Questionnaire (FPQ)[29, 44] measures fear and anxiety associated with pain. The FPQ is composed of 30 items divided into 3 subscales (severe pain, minor pain, and medical pain). Respondents are requested to indicate how fearful they would be if they were experiencing the pain described in the items (e.g., "*Breaking your arm*"). Answers are scored from 0 "not fearful" to 5 "extreme fearful". (8) Rumination: To explore the way participants typically think about negative experiences and problems, we administered the Perseverative Thinking Questionnaire (PTQ)[10]. This questionnaire comprises 15 items. The item pool included three items for each of the characteristics of repetitive negative thinking: repetitive, intrusive, difficulty to disengage from, unproductive and

capturing mental capacity. Participants had to rate the extent to which the 15 statements applied to them, on a scale ranging from 0 “never” to 4 “almost always”.

2.7. Response definition and data analysis overview

2.7.1. Response definition of the startle modulation

Using PSPHA[8], 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. Each peak amplitude was scored by subtracting its baseline score (averaged EMG level between 1 and 20 ms after the probe onset). A startle response was rejected if the baseline period was contaminated with noise or if a voluntary blink occurred during a 1−20 ms time window after probe onset, also when no visual peak could be detected (i.e. non-response), the response was rejected. Participants who failed to reach elevated peak amplitudes compared with baseline on more than 50% of the trials were considered non-responders and were excluded from further analyses. A total of 5 participants (1 HC and 4 FM) were excluded because of the absence of reliable startle eyeblink responses. Hence, the statistical analysis of the psychophysiological measure was run on a total sample of 55 participants. To make data comparable between individuals, despite the inter-individual differences in physiological reactivity, raw scores were transformed to z-scores. Furthermore, T-scores (a linear transformation of the z-scores) were used in the figures, to avoid negative values on the Y-axis and for an optimized visualization of the startle data. Averages were calculated for responding during CS/GS movements and ITI separately for both groups.

2.7.2 Data analysis overview

Some preparatory analyses were necessary in order to test our main research questions: *First*, as a manipulation check, we carried out a series of repeated measures ANOVAs to confirm that before conditioning both CSs did not differ with respect to affective valence, arousal and control experienced when performing the movements, but that after acquisition and generalization the CS+ became more

negative, elicited more arousal, and less feelings of being in control than the CS-. Because we had clear a priori hypotheses, we further analyzed the data using planned comparisons. The effect size indication η_G^2 is reported for all omnibus ANOVA effects[1, 41], and Greenhouse-Geisser corrections were applied when appropriate (uncorrected degrees of freedom and corrected *p*-values are reported together with ε). *Second*, because successful differential fear acquisition is a prerequisite to test fear generalization, we checked whether participants reported more fear in response to the CS+ movement than to the CS- movement at the end of the (transfer-of-)acquisition training as compared to the beginning. Therefore, we defined a random intercept two-level linear regression model to analyze the test effects of stimulus type (CS+/CS-) on the change in average pain-related fear ratings during four repeated measurements (i.e., during the three blocks of acquisition (A1/A2/A3) and one block of the transfer-of-acquisition phase (T1)), nested within persons for both groups (FM and HC) (see online supplementary material for the detailed statistical model description). The effects included in this model were estimated simultaneously using the SAS procedure MIXED[20, 50]. The model explains 63% of the observed variance in participants' pain-related fear ratings (see Table S1). We chose to include a subject-specific random intercept in the model, given that 48% of the variance was due to differences among participants: $\sigma_\theta^2/(\sigma_\theta^2 + \sigma_\varepsilon^2)$. *Third*, we tested the acquisition effects in the startle eyeblink measures using a repeated measures ANOVA, that is, whether participants had elevated startle responses to the CS+ movement compared to the CS- movement during (transfer-of-) acquisition.

After these preparatory analyses, we proceeded to test our main hypotheses: Following Meulders et al.[31], we expected that 1) HC would show an immediate transfer of differential pain-related fear and expectancy learning, whereas FM patients would show an initial loss of differential learning due to increased CS- responding, 2) FM patients would show flatter generalization gradients than HC and that this is caused by higher responses to the GSs that are more similar to the original CS-. Testing extinction of fear generalization we expect that 3) FM would show resistance to extinction to unreinforced GSs, whereas generalized fear will subside quickly in HC. In order to test the *first*

hypothesis, we defined two two-level linear regression models (one for each dependent variable) with two random intercepts (1 for each stimulus type) including group (FM and HC), linear trend variable T_j (which equals 0, 1, 2, 3 for trials j = T1, T2, T3, T4), as well as the interaction between the linear trend and group. The dependent variables were ratings of pain-US expectancy and fear of movement-related pain (see online supplementary material for the detailed statistical model description). The effects included in this model were estimated simultaneously using the SAS procedure MIXED[20, 50]. Both models are able to predict participants' pain-US expectancy and fear of movement-related pain ratings (see Table S2) very well as they explain 84% and 83%, respectively, of the observed variance in ratings. Including random intercepts was necessary as models without random intercepts explain respectively only 25% and 18% of the variance in observed ratings. In other words, a substantial part of the observed variance in ratings is due to participant differences.

In order to test the *second and third hypothesis*, we defined two two-level linear regression models (one for pain-US expectancy and one for fear of movement-related pain) with a subject-specific random intercept including group (FM and HC), trial (first and last trial of the generalization phase), a centered linear trend variable T_k (which equals -3, -2, -1, 0, 1, 2, 3 for trials k = CS+, GS1, GS2, GS3, GS4, GS5, GS6, CS-) modeling the linear component of the generalization gradient together with a quadratic trend variable $(T_k)^2$ modeling the quadratic component of the generalization gradient, as well as the interaction terms between group and the linear (quadratic) trend variable (see online supplementary material for the detailed statistical model description). The inclusion of the quadratic trend is supported by the data as the models including a quadratic trend have a better balance between complexity and goodness-of-fit (i.e., they have lower values of the Bayesian information criterion (BIC))[45]. BIC values for the model including only the linear trend were higher than for the model including both the linear and the quadratic trend, for both dependent variables, respectively pain-US expectancy: 3914 *vs.* 3874, and fear of movement-related pain: 3414 *vs*. 3396. The effects included in this model were estimated simultaneously using the SAS procedure MIXED[20, 50]. Both models are able to predict participants' pain-US expectancy and fear of movement-related pain ratings (see Table S3) quite well as they explain 62% and 65%, respectively, of the observed variance in

ratings. Including random intercepts was necessary as models without random intercepts explain respectively only 22% and 15% of the observed variance in ratings. Hence, a substantial part of the observed variance in ratings is due to participant differences. For all multilevel models, follow-up contrasts were calculated to further test our a priori hypotheses. Note that we did not observe a generalization gradient in the startle eyeblink measures for neither of the groups; in order not to overload the results section these analyses are omitted. Finally, in order to test our *fourth hypothesis*, pain-US intensity and unpleasantness ratings were analyzed using repeated measures ANOVAs to test whether pain would be more intense and more unpleasant for FM than for HC.

We decided to use a mixed analysis strategy combining both the multilevel modeling approach and the standard mixed RM ANOVA because unlike standard mixed RM ANOVA the multilevel approach (a) can model a quadratic trend, (b) it supports the estimation of a large variety of planned contrasts (i.e. all contrasts that can be expressed as a linear function of the underlying regression parameters), and (c) it supports the inclusion of random coefficients (e.g. random intercepts). Therefore we used the multilevel approach for the analyses where a more flexible approach involving these aspects was needed and more complexity also led to a better model fit. Consequently, multilevel modeling was used to conduct the manipulation check on fear acquisition in the self-reports and for testing hypothesis 1, 2 and 3. In contrast, for the manipulation checks on affective valence, arousal, control experienced, and the fear acquisiton in the startle measures, as well as to test hypothesis 4, a more standard mixed RM ANOVA turned out be satisfactory.

3. Results

3.1 Pain-US characteristics and questionnaires

In contrast with Meulders et al.[31], FM patients did not select a higher pain-US intensity during the calibration phase than the HC group, $t(58) = 1.54$, $p = .13$. There were also no differences in how the selected stimulus was subjectively rated by the FM group and the HC group during the calibration, $t(58) = 1.40$, $p = .17$, (see Table 1). For the analyses of the questionnaires, one FM patient was excluded, because she failed to complete any of the questionnaires. Independent *t*-tests were

conducted using the Holm-Bonferroni method to correct for multiple comparisons. The Holm-Bonferroni [18]correction is based on ranking the *p*-values of one family of hypotheses from smallest to largest. m is the number *p*-values. If the first *p*-value is greater than or equal to α/m, the procedure is stopped and no *p*-values are significant. If the first *p*-value is significant the second *p*-value is compared to α/(m-1), etc. Corrected *p*-values are reported. As expected, the groups had significantly different scores on most of the psychological trait questionnaires (see Table 3). In comparison with the HC group, FM patients reported higher pain intensity, $t(57)=14.65$, $p<.0001$, greater pain disability, $t(57)=14.66$, $p<.0001$, and more days of being disabled during the last 6 months, $t(57)=3.61$, $p<.01$, (P-GRAD-S). The FM group had significantly higher scores on the Catastrophizing, $t(57)=4.10$, $p<.01$, and the Limitation, $t(57)=8.05$, $p<.0001$, subscales, but no significant differences on the Optimism, $t(57)=-1.79$, $p=.48$, and Trust, $t(57)=-1.23$, $p=1.00$, and Internal Control, $t(57)=-2.02$, $p=.33$) subscales of the PCL in comparison with HC. FM reported more fear of movement and (re)injury, $t(57)=3.51$, $p<.01$, (TSK), reported more impairment in their daily life activities due to pain, $t(57)=13.56$, $p<.0001$, (FIQ), lower positive affect, $t(57)=-5.90$, $p<.0001$, and higher negative affect, $t(57)=5.30$, $p<.0001$, (PANAS). The FM group significantly differed on the Anxiety, $t(57)=-3.13$, $p<.05$), but not on the Depression subscale of the HADS, $t(57)=-1.15$, $p=.76$, nor did they differ with respect to general fear associated with pain as compared to the HC group, $t(57)=-0.24$, $p=.81$, (FPQ). Furthermore, no differences were found with respect to the FPQ subscales Medical pain, $t(57)=-0.86$, $p=.79$, and Severe pain, $t(57)=-1.15$, $p=1.00$, but FM patients tended to have higher scores for the Minor Pain subscale, $t(57)=2.82$, $p=.05$. FM patients also reported more repetitive negative thinking, $t(57)=4.09$, $p<.0001$, than the HC group (PTQ).

3.2 Manipulation checks

For all three SAM ratings, we performed a 2 (Group: FM/HC) x 2 (Stimulus Type: CS+/CS-) x 3 (Phase: Practice/Acq/Gen) mixed RM ANOVA.

3.2.1 Affective valence of the CS movements

This analysis revealed significant main effects for Group, $F(1, 58) = 4.80$, $p<.05$, $\eta_G^2=.03$, Stimulus Type, $F(1, 58) = 37.19$, $p<.001$, $\eta_G^2=.13$, and Phase, $F(2,116) = 5.99$, $p<.01$, $\varepsilon=.94$, $\eta_G^2=.03$. Further there was a significant Stimulus Type x Phase interaction, $F(2, 116) = 26.96$, $p<.0001$, $\varepsilon=.92$, $\eta_G^2=.09$, suggesting that the differences between the CS+ and the CS- changed over the experimental phases, this interaction was not modulated by Group, $F<1$. Planned comparisons further revealed that ratings for the CS+ and the CS- did not significantly differ at the end of the practice phase, $F<1$, but after acquisition, participants felt happier when performing the CS- movement than when performing the CS+ movement, $F(1, 58) = 46.57$, $p<.001$. This difference was still significant after generalization, $F(1, 58) = 44.61$, $p<.001$. Interestingly, there was no difference between the HC and the FM after acquisition and generalization with regards to the affective valence of the painful CS+ movement, $F(1, 58) = 1.08$, $p=.30$, but FM patients were more unhappy when performing the safe CS- movement, $F(1, 58) = 7.45$, $p<.01$.

3.2.2 Arousal elicited by the CS movements

This analysis showed a significant main effect for Stimulus Type, $F(1, 58) = 24.12$, $p<.001$, $\eta_G^2=.05$. Further there was a significant Stimulus Type x Phase interaction, $F(2, 116) = 6.52$, $p<.01$, $\varepsilon=.78$, $\eta_G^2=.02$, suggesting that the differences in arousal elicited by the CS+ and the CS- changed over the experimental phases, this interaction was not modulated by Group, $F<1$. Also the Phase x Group interaction was significant, $F(2, 116) = 5.69$, $p<.01$, $\varepsilon=.84$, $\eta_G^2=.03$, indicating that there was a difference between the groups regarding the arousal experienced during the different phases. Planned comparisons further confirmed that there were no differences in arousal elicited by the CS+ and the CS- at the end of the practice phase in both groups, Fs<1. Interestingly, FM patients reported being more aroused when performing both joystick movements before the conditioning procedure than the HC, $F(1, 58) = 5.34$, $p<.05$. After acquisition however, participants in both groups reported more arousal when performing the CS+ movement than when performing the CS- movement, FM: $F(1, 58) = 5.00$, $p<.05$; HC: $F(1, 58) = 11.25$, $p<.01$. This difference was still significant after generalization in both groups, FM: $F(1, 58) = 11.61$, $p<.01$; HC: $F(1, 58) = 8.99$, $p<.01$.

3.2.3 Feelings of being in control when performing CS movements

This analysis yielded significant main effects for Stimulus Type, $F(1, 58) = 23.48$, $p<.001$, $\eta_G^2=.05$, and Phase, $F(2, 116) = 10.21$, $p<.001$, $\varepsilon=.80$, $\eta_G^2=.05$. Further there was a significant Stimulus Type x Phase interaction, $F(2, 116) = 10.19$, $p<.001$, $\varepsilon=.86$, $\eta_G^2=.05$, suggesting that the differences in control experienced during the CS+ and the CS- changed over the experimental phases, this interaction was not modulated by Group, $F<1$. The Phase x Group interaction also reached significance, $F(2, 116) = 5.81$, $p<.01$, $\varepsilon=.80$, $\eta_G^2=.03$, indicating that there was a difference between the groups regarding the control experienced during the different phases. Planned comparisons further confirmed that there were no differences in control experienced during the CS+ and the CS- at the end of the practice phase in both groups, HC: $F<1$; FM: $F(1, 58) = 3.99$, $p=.051$. After acquisition however, participants in both groups reported feeling more in control when performing the CS- movement than when performing the CS+ movement, FM: $F(1, 58) = 16.67$, $p<.001$; HC: $F(1, 58) = 11.07$, $p<.01$. This difference was still significant after generalization in both groups, FM: $F(1, 58) = 14.86$, $p<.001$; HC: $F(1, 58) = 12.74$, $p<.001$.

3.2.4 Acquisition of self-reported fear of movement-related pain

Self-reported fear of movement-related pain acquisition was tested using a multilevel regression model (see online supplementary material for the detailed statistical model description). Table S1 (see online supplementary material) presents the results of the multilevel regression model for fear of movement-related pain ratings during (transfer-of-)acquisition, follow-up planned contrasts are visualized in Figure 2. When reporting planned contrasts $\hat{S}_l^{(k)}$ indicates the estimated slope for stimulus *k* (CS+, CS-) in group *l* (HC, FM) and $\hat{Y}_j^{(k)}(l)$represents the predicted rating for stimulus *k* (CS+, CS-) at block *j* (A1, A2, A3, T1) in group *l* (HC, FM).

There was a significant effect of time on the acquisition of fear of movement-related pain, indicating successful fear acquisition to the CS+, but not to the CS- (see Figure 2). This was indicated

by a significant difference in slopes for the CS+ and the CS- in both groups ($\hat{S}_{FM}^{(+)} - \hat{S}_{FM}^{(-)} = 0.93$, p < .0001; $\hat{S}_{HC}^{(+)} - \hat{S}_{HC}^{(-)} = 0.55$, $p < .05$). Differential fear of movement-related pain was already acquired at the first rating moment A1 (that is, after four movements of each type) in the HC, that is, they reported higher fear in response to the CS+ than the CS- ($\gamma_1 = 1.26$, $p < .01$), whereas this was not the case for the FM ($\gamma_2 = 0.76$, $p = .06$). At the last rating moment T1 (after the transfer-of-acquisition phase) the fear elicited by the CS+ was significantly higher compared with the CS- movement in both groups ($\gamma_3 = 2.90$, $p < .0001$; $\gamma_4 = 3.54$, $p < .0001$). These results indicate that participants in both groups learned to be afraid of the CS+ movement, but not the CS- movement; however, this differential learning was acquired slower by the FM than the HC.

3.2.5 Acquisition of fear-potentiated eyeblink startle

A 2 (Group: FM/HC) x 3 (Stimulus Type: CS+/CS-/ITI) x 4 (Block: A1-A3, T1) mixed RM ANOVA was carried out to test acquisition of differential fear learning in the eyeblink startle measures (see Figure 3). The results showed a significant main effect for Block, $F(3, 159) = 3.03$, $p<.05$, $\varepsilon=.92$, $\eta_G^2=.02$, indicating habituation, that is, startle responses declined gradually over time, but increased again during the transfer-of-acquisition phase probably because the change in procedure elicited an orientation response. Importantly, there was a significant main effect of Stimulus Type, $F(2, 106) = 4.11$, $p<.05$, $\varepsilon=.94$, $\eta_G^2=.02$. The Block x Stimulus Type interaction however just failed to reach significance, $F(6, 318) = 2.16$, $p=.05$, $\varepsilon=.89$, $\eta_G^2=.02$. The main effect of Group and all the interactions with this variable were not significant. Planned comparisons further confirmed that in both groups, the mean startle eyeblink amplitudes were elevated during the CS+ movement as compared to the CS- movement, $F(1, 53) = 10.95$, $p<.01$. These data confirm that participants in both groups successfully acquired similar levels of differential eyeblink startle responding.

3.3 Testing our primary hypotheses

<u>Hypothesis 1</u>: Differences in transfer-of-acquisition between healthy controls and fibromyalgia patients for pain-US expectancy and fear of movement-related pain ratings

Table S2 (see online supplementary material) presents the results of the multilevel regression model for the pain-US expectancy and fear of movement-related pain ratings during the four trials of the transfer-of-acquisition phase, follow-up planned contrasts are depicted in Figure 4. When reporting planned contrasts $\hat{Y}_j^{(k)}(l)$ is used to indicate the predicted rating for stimulus *k* (CS+, CS-) at trial *j* (T1, T2, T3, T4) in group *l* (HC, FM). At T1 (the first trial of the transfer-of-acquisition phase, see Figure 4a) HC still expected the pain-US to occur more after the CS+ than after the CS- ($\gamma_5 = 3.37$, $p<.0001$). In contrast with our expectations, FM also showed differential pain-US expectancies for the CS+ and the CS- at T1 ($\gamma_6 = 1.61$, $p<.05$), but the transferred CS+/CS- difference was not significantly smaller in the FM than the HC ($\gamma_{6-}\gamma_5 = -1.76$, $p=.07$). At T4, both groups showed stable differential pain-US expectancies for the CS+ and the CS- (HC: $\gamma_7 = 4.47$, $p<.0001$; FM: $\gamma_8 = 2.88$, $p<.0001$).

Interestingly, the pain-US expectancies in response to the CS+ did not differ between both groups ($\gamma_{9,}$ $\gamma_{10,}\gamma_{11,}\gamma_{12}$) at any of the transfer-of-acquisition trials, but FM expected the pain-US to occur more when performing the CS- than the HC, T1: $\gamma_{13} = 2.06$, $p<.01$; T2: $\gamma_{14} = 1.88$, $p<.01$; T3: $\gamma_{15} = 1.70$, $p<.01$; T4: $\gamma_{16} = 1.53$, $p<.05$, which is indicative of fragile safety learning (see Figure 4b).

Similarly, for the fear of movement-related pain ratings, at T1 (the first trial of the transfer-of-acquisition phase, see Figure 4c) HC reported to be more afraid to perform the CS+ movement than the CS- movement ($\gamma_{17} = 1.51$, $p<.01$). In line with our expectations, FM did not transfer the acquired CS+/CS- differential fear learning and thus were equally afraid to perform the CS+ and the CS- movement at T1 ($\gamma_{18} = 0.88$, $p=.09$). As expected, this lack of differentiation was due to elevated fear responses for the CS- in the FM as compared with the HC (T1: $\gamma_{19} = 1.71$, $p<.001$; T2: $\gamma_{20} = 1.53$, $p<.001$; T3: $\gamma_{21} = 1.35$, $p<.01$; T4: $\gamma_{22} = 1.18$, $p<.05$, whereas no such differences were observed for the CS+ ($\gamma_{23,}\gamma_{24,}\gamma_{25,}\gamma_{26}$). At T4, both groups reliably reported more pain-related fear to the CS+ than to the CS- (HC: $\gamma_{27} = 2.49$, $p<.0001$; FM: $\gamma_{28} = 2.76$, $p<.0001$) (see Figure 4d).

<u>Hypothesis 2</u>: Differences in generalization between healthy controls and fibromyalgia patients

Table S3 (see online supplementary material) presents the results of the multilevel regression model for the pain-US expectancy and fear of movement-related pain ratings during the first and the last trial of the generalization phase, follow-up planned contrasts are visualized in Figure 5. Note that when reporting contrasts $\hat{Y}_j^{(k)}(l)$ represents the predicted rating for stimulus *k* (CS+, GS1, GS2, GS3, GS4, GS5, CS-) at trial *j* (t1,t4) in group *l* (HC, FM). The generalization effect is expected to be the largest at trial 1, and to extinguish in the following trials, therefore the difference in generalization between the HC and FM patients is assessed at trial 1 and differences in extinction of generalization are assessed at trial 4 (and differences from trial 1 to trial 4). As predicted, there was a significant difference in the slope of the linear trend at the first trial of the generalization phase between both groups, $\beta^{(1)}_{LINxG}$ = 0.25, p<.05, indicating that FM showed flatter pain-US expectancy generalization gradients as compared with the HC. Planned comparisons (see Figure 5a) further confirmed that this difference in steepness of the slopes is explained by differences at the CS- side of the generalization gradient (i.e. responses to novel movements that are more similar to the safe movements), but that no differences occurred at the CS+ side of the generalization gradient (i.e. responses to novel movements that are more similar to the painful movements). More particularly, FM reported significantly higher pain-US expectancies for the CS- (γ_{29} = 2.23, p<.01), and GS5 (the generalization movement that was most similar to the CS-), γ_{30} = 1.46, p<.05, than the HC; the pain-US expectancy ratings for the other GSs and the original CS+ however did not differ between both groups ($\gamma_{31}, \gamma_{32}, \gamma_{33}, \gamma_{34}, \gamma_{35}$).

A similar data pattern was observed in the fear of movement-related pain ratings, however the statistical analyses did not fully corroborate our findings in the pain-US expectancy ratings. The linear trend variable did not interact with group, $\beta^{(1)}_{LINxG}$ = -.02, p=.78, suggesting that the steepness of the slopes for the HC and FM did not significantly differ for the fear of movement-related pain ratings. The quadratic trend tended to be different in both groups, but this difference was not statistically significant, $\beta^{(1)}_{QUADxG}$ = .08, p=.08. Planned contrasts (see Figure 5b) further showed that FM were more afraid of the original CS- (γ_{36} = 1.27, p<.05), than HC, but they also reported more fear of movement-

related pain in response to the original CS+ (γ_{37} = 1.40, p<.05); these elevated fear responses seemed to spread on both sides of the generalization gradient, but failed to reach significance (GS1: γ_{38} = 1.00, p=.07, and GS5: γ_{39} = 0.91, p=.09). The fear of movement-related pain ratings for the other GSs did not differ between both groups (γ_{40}, γ_{41}, γ_{42}).

Taken together, these results provide partial evidence for our hypothesis: pain-US expectancy generalization gradients are flatter in FM than HC due to elevated pain-US expectancies for the technically safe movements whereas the shape of the fear generalization gradient is not significantly different between FM and HC, but fear responses seem to be elevated on both sides of the continuum in FM.

<u>Hypothesis 3</u>: Differences in extinction of generalization between healthy controls and fibromyalgia patients

Predicted ratings of pain-US expectancy and fear of movement-related fear are depicted in Figure 5. At trial 4, there was a significantly different slope in the pain-US expectancy ratings for the FM *vs.* HC, $\beta^{(4)}_{LINxG}$ = 0.22, p<.05. Planned contrasts (see Figure 5c) further showed that pain-US expectancies in response to all GSs decreased significantly from trial 1 to trial 4 for the HC (γ_{43} = 1.47, γ_{44} = 2.41, γ_{45} = 2.77, γ_{46} = 0.35, γ_{47} = 1.71; all ps<.0001), but not for the CS+ (γ_{48} = -0.06, p=.91), that remained reinforced during the generalization phase, and not for the CS- (γ_{49} = 0.30, p=.56) (i.e. floor effect). The pain-US expectancy ratings for the FM also declined significantly from trial 1 to trial 4 for GS2 (γ_{50} = 1.19), GS3 (γ_{51} = 1.42), GS4 (γ_{52} = 1.36), and GS5 (γ_{53} = 1.03; all ps<.01) but not for the CS+ (γ_{54} = -0.10, p=.84), the CS- (γ_{55} = 0.42, p=.42), and the GS1 (γ_{56} = 0.68, p=.05). Interestingly, the decline in pain-US expectancies for GS2 ($\gamma_{56} - \gamma_{43}$ = -1.22), GS3 ($\gamma_{50} - \gamma_{44}$ = -1.35), and GS4 ($\gamma_{51} - \gamma_{45}$= -1.17; all ps<.05), was significantly smaller in the FM group than the HC, suggesting that there was resistance to extinction of pain-US expectancies to the novel, unreinforced generalization movements. At trial 4, the pain-US expectancies for all GSs (GS1: γ_{57} = 1.26, p<.05; GS2: γ_{58} = 1.64, p<.01; GS3: γ_{59} = 1.91, p<.001; GS4: γ_{60} = 2.08, p<.001; GS5: γ_{61} = 2.15, p<.0001), and the CS- (γ_{62}= 2.11, p<.01),

were indeed still significantly higher for the FM than the HC, which further supports the resistance to extinction of generalization hypothesis (see Figure 5a).

A similar analysis was run on the fear of movement-related pain ratings, however this analysis could only partly confirm our findings in the pain-US expectancy ratings. At trial 4, there was no significantly different slope in fear of movement-related pain ratings for the FM *vs.* HC, $\beta^{(4)}_{LINxG}$ = -0.05, p=.51. Planned contrasts (see Figure 5d) further showed that fear in response to all GSs decreased significantly from trial 1 to trial 4 for the HC, (GS1: γ_{63} = 0.66, p<.05; GS2: γ_{64} = 0.98, p<.001; GS3: γ_{65} = 1.09, p<.0001; GS4: γ_{66} = 1.00, p<.001; GS5: γ_{67} = 0.70, p<.01), but not for the CS+ (γ_{68} = 0.15, p=.69), that remained reinforced during the generalization phase, and not for the CS- (γ_{69} = 0.22, p=.57) (i.e. floor effect). The fear of movement-related pain ratings for the FM also declined significantly from trial 1 to trial 4 for GS2 (γ_{70} = 0.73, p<.01), GS3 (γ_{71} = 0.86, p<.01), GS4 (γ_{72} = 0.81, p<.01),and GS5 (γ_{73} = 0.59, p<.05), but not for the CS+ (γ_{74} = -0.06, p=.87), the CS- (γ_{75} = 0.18, p=.63), and the GS1 (γ_{76} = 0.42, p=.10). The decline in fear of movement-related pain was not significantly smaller in the FM group than the HC (see Table 9). At trial 4, however the fear of most generalization movements was still significantly higher for the FM than the HC, (GS1: γ_{77} = 1.24, p<.01; GS2: γ_{78} = 0.99, p<.05; GS3: γ_{79} = 0.87, p=.06; GS4: γ_{80} = 0.89, p=.05; GS5: γ_{81} = 1.03, p<.05), which at least provides partial support for the resistance to extinction of generalization hypothesis (see Figure 5c).

<u>Hypothesis 4</u>: Differences in pain-US intensity and unpleasantness between healthy controls and fibromyalgia patients

We examined the differences in self-reported intensity and unpleasantness of the pain-US by performing 2 mixed RM ANOVAs including Group (FM/HC) and Block (A1-A3, T1, GEN). These analyses yielded significant main effect of Group (unpleasantness: $F(1, 58) = 4.91$, p<.05, η^2_G=.10 intensity: $F(1, 58) = 8.26$, p<.05, η^2_G=.06). The main effect and the interaction with Block failed to

reach significance in both analyses. These results confirm that FM rated the selected pain-US as more painful and more unpleasant throughout the experiment than the HC.

4. Discussion

To our knowledge, this is the first study that investigated the differences in generalization gradients of pain-related fear and expectancy between fibromyalgia patients (FM) and age- and gender-matched healthy pain-free controls (HC), and subsequently compared the rate of extinction of generalization between both groups. We hypothesized that FM would show (1) poorer transfer of safety learning to a novel context, (2) flatter generalization gradients compared to HC due to higher responses to the GSs that are more similar to the original CS-, (3) impaired extinction of unreinforced GSs, whereas generalized pain-related fear and expectancy will subside quickly in HC, and (4) higher levels of pain unpleasantness and intensity than HC.

The results can be summarized as follows: *First*, we successfully established acquisition of fear of movement-related pain in both groups. This effect was evident by elevated startle amplitudes, higher pain-related fear and expectancy ratings for the CS+ than for the CS-. Participants also felt unhappier, less in control and more aroused while performing the CS+ movement compared to the CS- movement. Interestingly, whereas HC acquired these CS-US contingencies after only one acquisition block, it took longer for FM to pick up on these relationships. *Second*, as predicted, FM showed poorer transfer of safety learning to a novel context than HC. During the transfer-of-acquisition phase, we switched from a voluntary to a signaled movement set-up, implying that participants needed to transfer the acquired CS-US contingencies to a novel context. HC did transfer these contingencies impeccably and showed differential fear responses to the CS+/- from the first trial on, but FM did not. These results seem to suggest that once adaptive differential fear learning is acquired, it is fragile and sensitive to context switches in FM. Furthermore, fear responses during the CS+ did not differ between groups, but CS- responses were elevated in FM compared with HC, indicating disruptive safety learning. This pattern was not completely mirrored in the pain-US expectancy measures; no loss of transfer of differential learning was observed. Nevertheless, again pain-US expectancies in response

to the CS- were elevated for FM as compared with HC, whereas no such differences were observed for the CS+. These findings also provide evidence for the fragility of safety learning in FM. The vulnerability of safety learning can be understood in terms of the associative learning theory. That is, safety learning can be seen as inhibitory learning to the CS-. In contrast to excitatory learning to the CS+, which generalizes easily to new contexts, safety learning to the CS- is a form of inhibitory learning like extinction learning, which is a more fragile learning process that is under contextual control. Nowadays, extinction is commonly viewed as acquiring a new CS-noUS association that inhibits the behavioral expression of the first learned association rather than the forgetting/overwriting of the original CS-US association[3]. As a consequence, which of both co-existing associations controls behavior is context-dependent. The effect observed in our study is very similar to renewal (i.e. a return of fear after successful extinction due to a context switch) because the acquired safety learning disappears when a context change occurs (i.e. signaled versus voluntary movement set-up). These findings corroborate previous findings of Meulders et al.[31], and suggest that FM, who seem to be characterized by fragile safety learning, might have difficulties transferring CS-noUS contingencies to other contexts. *Third*, with respect to fear generalization, we replicated and extended our previous findings using a design in which the GSs either had a feature in common with the CS+ or CS- but no generalization gradients could be calculated[31]. More specifically, we showed that pain expectancy generalization gradients are flatter in FM than in HC due to elevated pain expectancies for the novel, technically safe movements, whereas the shape of the fear generalization gradient did not differ between both groups. These findings are also in line with our study on pain expectancy judgments in chronic hand pain patients[30]. *Fourth,* with respect to extinction of generalization, we found at least partial evidence for our hypothesis: we showed that although the pain expectancy for all generalization movements declined for HC and for all but the GS1 in FM, this decline was still significantly smaller in FM than in HC. Moreover after four unreinforced trials, pain expectancies for all generalization movements remained elevated for FM compared to HC. A similar pattern was observed in the pain-related fear ratings, fear in response to all generalization movements declined for the HC and for all but the GS1 in the FM, this decline was however was not significantly different in FM than in HC.

Nevertheless, after four unreinforced trials, pain-related fear of most of the generalization movements remained elevated for the FM compared to the HC. These results suggest a deficiency in the extinction of fear generalization in FM patients, which may contribute to the maintenance of chronic disability in patients. Closely related, Flor and colleagues previously demonstrated that, relative to HC, chronic back pain patients showed similar rates of acquisition, but slower extinction of verbal as well as cortical pain responses[13]. The current findings are also in line with previous research on fear extinction in anxiety disorder patients. For example, Wessa and Flor[56] reported that PTSD patients have a deficit in extinction of traumatic response. Michael et al.[39] conducted a study with panic disorder (PD) patients and found that PD patients too showed impaired extinction learning. The current findings corroborate previous research in anxiety disorders that found differences in fear extinction between HC[17, 40] and, essentially extend these findings by showing impaired extinction of generalization. Previous research also showed that safety learning is particularly vulnerable in individuals with high trait anxiety and relatively low levels of positive affect[32]. In the present study, FM scored relatively high on trait anxiety, and low on positive affect, and showed fragile safety learning, which further corroborates previous findings. *Fifth*, FM tended to select a lower intensity, however, the physical intensity did not significantly differ from HC. FM rated the selected pain-US as more intense and more unpleasant than HC, which might be due to increased pain sensitivity, corroborating previous observations[42].

There are some limitations that should be addressed as well. *First*, five participants were excluded from the startle analyses. Due to reduced statistical power, general interactions might have failed to reach statistical significance. *Second*, only one male participated in our study, so the results cannot necessarily be generalized to a male population. However, women are affected with FM about three times more often than men[28]. Therefore, it can be argued that our sample composition is justified. *Third*, no conclusions can be drawn about the causal relationship between impaired safety learning and overgeneralization of pain-related fear in FM, because we did not use a longitudinal design, which is needed to draw such conclusions. Future research might use longitudinal designs to investigate the causal relationship of fear learning deficits in the origin and maintenance of FM. *Fourth*, the groups

also differed with respect to medication use[6, 11, 16] and comorbidity with anxiety and depression[21, 26, 27] thus we cannot exclude the possibility that this might have contributed to the observed differences in fear learning/expression. Indeed, impaired safety learning[27], fear overgeneralization[25, 26] and resistance to extinction[39, 56] has been reported in anxiety disorder patients as well. Moreover, anxiolytics might affect the expression of context conditioning[15], opioids have shown to impair fear learning[11], and antidepressants may enhance cued fear conditioning[7, 16]. 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.

Fear generalization research explains how stimuli that were never associated with pain themselves may trigger fear. From a clinical perspective, an extensive analysis of crucial stimuli and their conditioning history should be fed back into exposure treatment –the clinical analogue of Pavlovian extinction and golden standard for fear reduction. Exposure treatment often involves GSs because the original CSs are unavailable/inaccessible. Previous research has shown that extinction of the original CS spreads to GSs[51], but not necessarily the other way around. The observation that FM show slower extinction of generalized fear may thus be especially problematic for exposure treatment responsivity. A plausible way to overcome this deficit, is to use a broad array of GSs (by analogy of exposure in different contexts to promote the generalization of extinction)[9].

To conclude, this study showed poorer transfer of safety learning to a novel context in FM than HC. Further, results provided partial evidence for our overgeneralization hypothesis: pain expectancy generalization gradients were flatter in FM than HC due to elevated pain expectancies for the technically safe movements whereas the shape of the fear generalization gradient was not different for FM and HC. Fearful responding declined to the generalization movements in both groups, but extinction of generalization of pain-related fear and expectancy was impaired in FM as compared to HC. We contend that this failure of extinction of generalization might be a contributing factor in the exacerbation and maintenance of fibromyalgia syndrome pathology and disability.

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6. Conflict of interest Statement

The authors report no conflict of interest

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Figure Captions

Figure 1. Schematic overview of the experimental task during the generalization phase.

Figure 2. Predicted difference of fear of movement-related pain ratings in response to CS+ and CS- movements during acquisition (A1-3) and transfer-of-acquisition (T) for both groups (HC and FM).

Figure 3. Mean eyeblink startle amplitudes (±SE's) during the CS movements and the ITI during (transfer-of -)acquisition. Note – that for graphic purposes T-scores were used.

Figure 4. Predicted difference in (a) pain-US expectancy ratings (CS+ minus CS-) for both groups (HC and FM), (b) pain-US expectancy ratings (FM minus HC) for both CSs, (c) fear of movement-related pain ratings (CS+ minus CS-) for both groups (HC and FM), and (d) fear of movement-related pain ratings (FM minus HC) for both CSs during the four trials of the transfer-of-acquisition (T1-4).

Figure 5. Predicted difference in (a) pain-US expectancy ratings (FM minus HC) at the first trial (T1) and last trial (T4) of the generalization phase, (b) fear of movement-related pain ratings (FM minus HC) at the first trial (T1) and last trial (T4) of the generalization phase, (c) pain-US expectancy ratings (T1 minus T4) for both groups (HC and FM), and (d) fear of movement-related pain ratings (T1 minus T4) for both groups (HC and FM).

Table 1. *Demographic and clinical characteristics for the fibromyalgia (FM) group (n=30) and the healthy control (HC) group (n=30) separately.*

Total *N* = 60	FM group		HC group			
	Mean	*SD*	*Mean*	*SD*	*t*	*df*
Selected pain intensity level (in mA)	18.73	8.53	22.90	12.18	1.54	58
Selected self-reported pain intensity (range 1-10)	7.87	0.35	7.98	0.18	1.40	58
Age (in years)	40.80	11.28	40.63	11.98	-0.06	58
WPI (range 0-19)	9.43	3.89				
SS (range 0-12)	8.13	1.66				
Highest education level						
Primary school	10%		0%			
Vocational secondary education	23%		0%			
Technical secondary education	17%		10%			
General secondary education	7%		13%			
Professional bachelor's degree	10%		10%			
Academic bachelor's degree	20%		47%			
Master's degree	3%		17%			
Other	10%		3%			
Type of medication						
Antidepressants	40%		0%			
Anxiolytics	20%		0%			
Analgesics (opioids)	33%		0%			
Analgesics (non-opioids)	43%		0%			

Other	47%	7%

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: muscle relaxants, hormones, anti-hypertension, antiarrhythmic, gastric ulcer medication, dopamine, synthetic thyroid hormone, psoriasis medication, magnesium supplements, and probiotics.

Table 2. *Study design summary*

Practice	**Habituation**	**Acquisition**	**Transfer-of-acquisition**	**Generalization**
(2 x 8 trials)	**(8 trials)**	**(3 x 8 trials)**	**(1 x 8 trials)**	**(4 x 7 trials)**
2 x [4 x CS+ only]	8 probes	3 x [4 x CS+]	4 x CS+	4 x CS+
2 x [4 x CS-]		3 x [4 x CS-]	4 x CS-	4 x CS-
				4 x GS1-5

Note – CS = conditioned stimulus (movement quadrant 1 and 7); GS = generalization stimulus (movement quadrants 2-6); pain-US = painful electrocutaneous stimulus (2 ms duration); CS+ and CS-, respectively, refer to the movement direction that is followed by the pain-US (75% reinforcement) and the movement that is never followed by the pain-US. The suffix "only" is used to indicate non-reinforcement of the CS+ movement (i.e. during the practice phase). GS movements are never reinforced. Both groups (FM and HC) were subjected to the same experimental procedure.

Table 3. *Questionnaire scores for the fibromyalgia (FM) group and healthy control (HC) group separately.*

Total sample *N*=59	FM group (*n* = 29)		HC group (*n* = 30)			
	Mean	*SD*	*Mean*	*SD*	*t*	*df*
CPGS – pain intensity*	65.29	14.18	15.56	11.82	14.65	57
CPGS – pain disability*	56.90	17.36	5.22	8.29	14.66	57
CPGS – number of days disability*	44.90	66.47	1.00	2.85	3.61	57
PCL – catastrophizing*	44.17	13.36	30.23	12.75	4.10	57
PCL – limitation*	27.14	5.12	16.20	5.31	8.05	57
PCL – optimism	23.86	4.31	26.37	6.26	-1.79	57
PCL – internal control	16.1	4.18	18.20	3.77	-2.02	57
PCL – trust	13.41	2.82	14.40	3.32	-1.23	57
TSK – total score*	41.07	7.93	34.33	6.80	3.51	57
FIQ – total score*	55.35	15.15	13.82	7.09	13.56	57
PANAS – positive affect*	30.45	7.87	40.43	4.82	-5.90	57
PANAS – negative affect*	24.14	8.53	14.97	4.06	5.30	57
HADS – anxiety*	17.72	3.24	19.90	1.97	-3.13	57
HADS – depression	15.45	2.23	16.00	1.36	-1.15	57
FPQ – medical pain	18.90	6.76	20.27	5.41	-0.86	57
FPQ – minor pain	16.72	4.98	13.67	3.19	2.82	57
FPQ – severe pain	24.14	8.36	26.73	8.91	-1.15	57

FPQ – total score	59.76	16.30	60.67	13.16	-0.24	57
PTQ – total score *	27.76	13.79	14.30	11.42	4.09	57

Note – **p*<.05, after Holm-Bonferroni corrections. One patient failed to fill out the questionnaires. Based on the CPGS scales, 10% (3/29) of the fibromyalgia patients was classified as Grade I (low disability - low intensity), 28% (8/29) as Grade II (low disability - high intensity), 14% (4/29) as Grade III (high disability - moderately limiting) and 48% (14/29) as Grade IV (high disability - severely limiting).

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 of Tampa Scale of Kinesiophobia; FIQ = total score of 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; FPQ = total score of Fear of Pain Questionnaire: subscales are calculated for medical pain, minor pain, and severe pain; PTQ = total score of Perseverative Thinking Questionnaire.

Table S1**.** *Results of the multilevel regression model predicting retrospective fear of movement-related pain ratings for CS+ versus CS- during the acquisition and transfer-of-acquisition phases for subjects in the control (HC) group versus fibromyalgia (FM) group*.

Coefficient	Description	Estimate	SE	*p*-value
$\mu^{(-)}$	Predicted intercept of the linear trend (i.e. at T=0) for CS- for HC with an average random intercept value (i.e. $\theta_i = 0$)	0.97	0.43	0.026
$\beta_T^{(-)}$	Slope of the linear trend of CS- for HC	-0.07	0.15	0.661
$\beta_G^{(-)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS- for HC *vs*. FM	1.10	0.61	0.072
$\beta_{TxG}^{(-)}$	Increase in the slope of the linear trend of CS- for HC *vs*. FM	-0.10	0.21	0.641
$\mu^{(+)}$	Predicted intercept of the linear trend (i.e. at T=0) for CS+ for HC with an average random intercept value (i.e. $\theta_i = 0$)	2.23	0.43	<.0001
$\beta_T^{(+)}$	Slope of the linear trend of CS+ for HC	0.48	0.15	0.002
$\beta_G^{(+)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS+ for HC *vs*. FM	0.60	0.61	0.328
$\beta_{TxG}^{(+)}$	Increase in the slope of the linear trend of CS+ for HC *vs*. FM	0.28	0.21	0.193
σ_θ^2	Variance of the subject-specific predicted ratings at block A1 (i.e. T=0)	3.16	0.67	<.0001
σ_ε^2	Variance of the error term	3.45	0.24	<.0001
R^2	Proportion of explained variance	62.8%		

Table S2**.** *Results of the multilevel regression models predicting fear of movement-related pain and pain-US expectancy ratings for CS+ versus CS- during the transfer-of-acquisition phase for subjects in the control (HC) group versus the fibromyalgia (FM) group.*

Regression model predicting pain-US expectancy				
Coefficient	Description	Estimate	SE	*p*-value
$\mu^{(-)}$	Predicted intercept of the linear trend (i.e. at T=0) for CS- for HC with an average random intercept value (i.e. $\theta_i^{(CS-)} = 0$)	2.550	0.459	<.0001
$\beta_T^{(-)}$	Slope of the linear trend of CS- for HC	-0.283	0.127	0.027
$\beta_G^{(-)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS- HC *vs*. FM	2.057	0.650	0.002
$\beta_{TxG}^{(-)}$	Increase in the slope of the linear trend of CS- HC *vs*. FM	-0.177	0.180	0.327
$\mu^{(+)}$	Predicted intercept of the linear trend (i.e. at T=0) for HC with an average random intercept value (i.e. $\theta_i^{(CS+)} = 0$)	5.917	0.570	<.0001
$\beta_T^{(+)}$	Slope of the linear trend of CS+ for HC	0.083	0.127	0.513
$\beta_G^{(+)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS+ for HC *vs.* FM	0.297	0.807	0.713
$\beta_{TxG}^{(+)}$	Increase in the slope of the linear trend of CS+ for HC *vs.* FM	-0.120	0.180	0.505
$\sigma^2_{(CS-)}$	Variance of the subject-specific predicted ratings for CS- at T1 (i.e. T=0)	4.633	0.974	<.0001
$\sigma^2_{(CS+)}$	Variance of the subject-specific predicted ratings for CS+ at T1 (i.e. T=0)	8.058	1.610	<.0001
σ^2_{ε}	Variance of the error term	2.428	0.182	<.0001
R^2	Proportion of explained variance	84.0%		
Regression model predicting fear of movement-related pain ratings				
Coefficient	Description	Estimate	SE	*p*-value
$\mu^{(-)}$	Predicted intercept of the linear trend (i.e. at T=0) for CS- for HC with an average random intercept value (i.e. $\theta_i^{(CS-)} = 0$)	1.270	0.361	0.001
$\beta_T^{(-)}$	Slope of the linear trend of CS- for HC	-0.247	0.112	0.029
$\beta_G^{(-)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS- for HC *vs.* FM	1.707	0.510	0.001

$\beta_{TxG}^{(-)}$	Increase in the slope of the linear trend of CS- for HC *vs.* FM	-0.177	0.159	0.266
$\mu^{(+)}$	Predicted intercept of the linear trend (i.e. at T=0) for CS+ for HC with an average random intercept value (i.e. $\theta_i^{(CS+)} = 0$)	2.780	0.549	<.0001
$\beta_T^{(+)}$	Slope of the linear trend of CS+ for HC	0.080	0.112	0.476
$\beta_G^{(+)}$	Increase in the intercept of the linear trend (i.e. at T=0) of CS+ for HC *vs.* FM	1.073	0.777	0.168
$\beta_{TxG}^{(+)}$	Increase in the slope of the linear trend of CS+ for HC *vs.* FM	0.123	0.159	0.437
$\sigma^2_{(CS-)}$	Variance of the subject-specific predicted ratings for CS- at T1 (i.e. T=0)	2.580	0.568	<.0001
$\sigma^2_{(CS+)}$	Variance of the subject-specific predicted ratings for CS+ at T1 (i.e. T=0)	7.733	1.524	<.0001
σ^2_{ε}	Variance of the error term	1.887	0.141	<.0001
R^2	Proportion of explained variance	82.7%		

Table S3. *Results of the multilevel regression models predicting pain-US expectancy and fear of movement-related pain ratings for the conditioned (CS+ and CS-) and generalization stimuli (GS1-5) at trial 1 and trial 4 of the generalization phase for subjects of the control group versus fibromyalgia group.*

Regression model predicting pain-US expectancy				
Coefficient	Description	Estimate	SE	*p*-value
$\mu^{(1)}$	Predicted rating at trial 1 for GS_3 (i.e. T=0) for HC (i.e. $G = 0$), and an average random intercept value (i.e. $\theta_i = 0$)	3.651	0.445	<.0001
$\beta^{(1)}_{LIN}$	Average change in the predicted rating at trial 1 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	-0.593	0.073	<.0001
$\beta^{(1)}_{QUAD}$	Half of the expected change in the slope of the linear trend for trial 1 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	-0.044	0.042	0.303
$\beta^{(1)}_{G}$	Average difference in the predicted rating at trial 1 for GS_3 (i.e. T=0) for HC *vs.* FM	0.560	0.629	0.373
$\beta^{(1)}_{LINxG}$	Difference in the slope of the linear trend at trial 1 for HC *vs.* FM	0.246	0.104	0.018
$\beta^{(1)}_{QUADxG}$	Half of the difference in the expected change in the slope of the linear trend at trial 1 for GS_k compared with GS_{k-1} for HC *vs.* FM	0.103	0.060	0.087
$\mu^{(4)}$	Predicted rating at trial 4 for GS_3 (i.e. T=0) for HC (i.e. $G = 0$), and an average random intercept value (i.e. $\theta_i = 0$)	0.884	0.396	0.029
$\beta^{(4)}_{LIN}$	Average change in the predicted rating at trial 4 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	-0.654	0.073	<.0001
$\beta^{(4)}_{QUAD}$	Half of the expected change in the slope of the linear trend at trial 4 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	0.250	0.042	<.0001
$\beta^{(4)}_{G}$	Average difference in the predicted rating at trial 4 for GS_3 (i.e. T=0) for HC *vs.* FM	1.910	0.560	0.0007
$\beta^{(4)}_{LINxG}$	Difference in the slope of the linear trend at trial 4 for HC *vs.* FM	0.220	0.104	0.034
$\beta^{(4)}_{QUADxG}$	Half of the difference in the expected change in the slope of the linear trend at trial 4 for GS_k compared with GS_{k-1} for HC *vs.* FM	-0.051	0.060	0.393
$\sigma^2_{(1)}$	Variance of the subject-specific predicted ratings on trial 1 for GS_3 (i.e. T=0)	4.428	0.943	<.0001

$\sigma^2_{(4)}$	Variance of the subject-specific predicted ratings on trial 4 for GS_3 (i.e. T=0)	3.193	0.714	<.0001
σ^2_{ε}	Variance of the error term	4.524	0.240	<.0001
R^2	Proportion of explained variance	61.6%		

Regression model predicting fear of movement-related pain

Coefficient	Description	Estimate	SE	*p*-value
$\mu^{(1)}$	Predicted rating at trial 1 for GS_3 (i.e. T=0) for HC (i.e. $G = 0$), and an average random intercept value (i.e. $\theta_i = 0$)	1.681	0.392	<.0001
$\beta^{(1)}_{LIN}$	Average change in the predicted rating at trial 1 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$).	-0.314	0.055	<.0001
$\beta^{(1)}_{QUAD}$	Half of the expected change in the slope of the linear trend for trial 1 for GS_k compared with stimulus GS_{k-1} for HC (i.e. $G = 0$).	-0.007	0.031	0.821
$\beta^{(1)}_{G}$	Average difference in the predicted rating at trial 1 for GS_3 (i.e. T=0) for HC *vs.* FM	0.644	0.555	0.246
$\beta^{(1)}_{LINxG}$	Difference in the slope of the linear trend at trial 1 for HC *vs.* FM	-0.021	0.077	0.781
$\beta^{(1)}_{QUADxG}$	Half of the difference in the expected change in the slope of the linear trend at trial 1 for GS_k compared with GS_{k-1} for HC *vs.* FM	0.077	0.045	0.084
$\mu^{(4)}$	Predicted rating at trial 4 for GS_3 (i.e. T=0) for HC (i.e. $G = 0$), and an average random intercept value (i.e. $\theta_i = 0$)	0.594	0.330	0.077
$\beta^{(4)}_{LIN}$	Average change in the predicted rating at trial 4 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	-0.325	0.055	<.0001
$\beta^{(4)}_{QUAD}$	Half of the expected change in the slope of the linear trend at trial 4 for GS_k compared with GS_{k-1} for HC (i.e. $G = 0$)	0.093	0.031	0.003
$\beta^{(4)}_{G}$	Average difference in the predicted rating at trial 4 for GS_3 (i.e. T=0) for HC *vs.* FM	0.871	0.466	0.062
$\beta^{(4)}_{LINxG}$	Difference in the slope of the linear trend at trial 4 for HC *vs.* FM	-0.051	0.077	0.507
$\beta^{(4)}_{QUADxG}$	Half of the difference in the expected change in the slope of the linear trend at trial 4 for stimulus GS_k compared with G_{k-1} HC *vs.* FM	0.065	0.045	0.142
$\sigma^2_{(1)}$	Variance of the subject-specific predicted ratings on trial 1 for GS_3 (i.e. T=0)	3.784	0.769	<.0001

$\sigma^2_{(4)}$	Variance of the subject-specific predicted ratings on trial 4 for GS_3 (i.e. T=0)	2.426	0.517	<.0001
σ^2_{ε}	Variance of the error term	2.497	0.132	<.0001
R^2	Proportion of explained variance	65.2%		

Multilevel regression model predicting retrospective fear of movement-related pain ratings for CS+ versus CS- during the acquisition and transfer-of-acquisition phases for subjects of the control group (HC) versus fibromyalgia (FM) group

To describe the model we assume that Y_{ijkl} represents the rating of fear of movement-related pain of person *i* (*i*=1,..,60) of group *l* (*l*=HC, FM) for stimulus *k* (*k*=CS-, CS+) after block *j* of the acquisition or transfer-of-acquisition phase (*j*=A_1, A_2, A_3, T_1). It is assumed that observed ratings change linearly at the subsequent blocks. In particular, the linear trend variable T_j (which equals 0, 1, 2, 3 for blocks j=A_1, A_2, A_3, T_1) is used to model a linear trend of the observed rating in the subsequent blocks. Furthermore, the variable G_l equals 0 for HC and 1 for FM.

The following multilevel regression model is used:

$$Y_{ijkl} = \theta_i + \mu^{(k)} + \beta_T^{(k)} T_j + \beta_G^{(k)} G_l + \beta_{TxG}^{(k)} T_j G_l + \varepsilon_{ijkl}$$

The model includes specific regression coefficients for each of the stimuli (k=CS-, CS+). Furthermore, the model includes a subject-specific random intercept $\theta_i \sim N(0, \sigma_\theta^2)$ to model subject differences in the observed ratings after block A_1, and an error term $\varepsilon_{ijkl} \sim N(0, \sigma_\varepsilon^2)$ to model unexplained noise in the data.

The parameters of the model should be interpreted as follows:

$\mu^{(k)}$= Predicted intercept of the linear trend (i.e. at T=0) of stimulus type k for subjects of HC with an average random intercept value.

$\beta_T^{(k)}$= Slope of the linear trend of stimulus type k for subjects of HC.

$\beta_G^{(k)}$=Increase in the intercept of the linear trend (i.e. at T=0) of stimulus type k for FM compared to HC.

$\beta_{TxG}^{(k)}$ = Increase in the slope of the linear trend of stimulus type for FM compared to HC.

σ_{θ}^{2} = Variance of the subject-specific predicted ratings at block A1 (i.e. T=0).

σ_{ε}^{2} = Variance of the error term.

Multilevel regression model predicting pain-US expectancy and fear of movement-related pain ratings for CS+ versus CS- during the transfer-of-acquisition phase for subjects of the control (HC) group versus fibromyalgia (FM) group

To describe the model we assume that Y_{ijkl} represents the rating (i.e. fear of movement-related pain or pain-US expectancy) of person *i* (*i*=1,..,60) of group *l* (*l*=HC, FM) for stimulus *k* (*k*=CS-, CS+) after trial *j* of the transfer phase (*j*=T_1, T_2, T_3, T_4). It is assumed that observed ratings change linearly at the subsequent trials. In particular, the linear trend variable T_j (which equals 0, 1, 2, 3 for trials *j*=T_1, T_2, T_3, T_4) is used to model a linear trend of the observed rating in the subsequent trials. Furthermore, the variable G_l equals 0 for subjects of HC and 1 for subjects of FM.

The following multilevel regression model is used:

$$Y_{ijkl} = \theta_i^{(k)} + \mu^{(k)} + \beta_T^{(k)} T_j + \beta_G^{(k)} G_l + \beta_{TxG}^{(k)} T_j G_l + \varepsilon_{ijkl}$$

The model includes specific regression coefficients for each of the stimuli (*k*=CS-, CS+). Furthermore, the model includes subject-specific random intercepts $\theta_i^{(k)}$ for each of the stimuli (*k*=CS-, CS+) to model subject differences in the observed ratings after trial T_1, and an error term $\varepsilon_{ijkl} \sim N(0, \sigma_{\varepsilon}^2)$ to model unexplained noise in the data. It is assumed that the random intercepts ($\theta_i^{(CS+)}$, $\theta_i^{(CS-)}$) have a multivariate normal distribution with mean (0,0) and a variance-covariance matrix that is estimated from the data.

The parameters of the model should be interpreted as follows:

$\mu^{(k)}$= Predicted intercept of the linear trend (i.e. at T=0) of stimulus type *k* for subjects of HC with an average random intercept value.

$\beta_T^{(k)}$= Slope of the linear trend of stimulus type k for subjects of HC.

$\beta_G^{(k)}$=Increase in the intercept of the linear trend (i.e. at T=0) of stimulus type *k* for FM compared to HC.

$\beta_{TxG}^{(k)}$= Increase in the slope of the linear trend of stimulus type *k* for FM compared to HC.

$\sigma_{(k)}^2$= Variance of the subject-specific predicted ratings at T_1 (i.e. T=0) for stimulus type *k*.

σ_ε^2= Variance of the error term.

Multilevel regression model predicting the generalization and extinction of generalization of pain-US expectancy and fear of movement-related pain ratings for subjects of the control group (HC) versus fibromyalgia (FM) group

To describe the model we assume that Y_{ijkl} represents the rating (i.e. fear of movement-related pain or pain-US expectancy) of person *i* (*i*=1,..,60) on trial *j* (*j*=trial 1, trial 4) of group *l* (*l*= HC, FM) for conditioned or generalization stimulus *k* (*k*=CS+, GS1, GS2, GS3, GS4, GS5, CS-). It is assumed that, for each trial, observed ratings change quadratically in the subsequent stimuli approaching from CS+ and CS-. The centered linear trend variable T_k (which equals -3, -2, -1, 0, 1, 2, 3 for stimuli *k*=CS+, GS1, GS2, GS3, GS4, GS5, CS-) is used to capture a linear component in the observed rating for the set of conditioned and generalization stimuli modeling the expected generalization gradient. Likewise, the quadratic component is modelled by including the quadratic variable $(T_k)^2$ in the model. The variable G_l equals 0 for subjects of HC and 1 for subjects of FM. To investigate whether the strength

of the linear (quadratic) component differs for HC versus FM, we include an interaction between G and the linear (quadratric) trend variable.

In particular, the following multilevel regression model is used:

$$Y_{ijkl} = \mu^{(j)} + \theta_i^{(j)} + \beta_{LIN}^{(j)} T_k + \beta_{QUAD}^{(j)} (T_k)^2 + \beta_G^{(j)} G_l + \beta_{LINxG}^{(j)} T_k G_l + \beta_{QUADxG}^{(j)} (T_k)^2 G_l + \varepsilon_{ijkl}$$

The model includes a subject-specific random intercept $\theta_i^{(j)}$ to model subject differences in the reported ratings for stimulus GS3 at trial *j*, and an error term $\varepsilon_{ijkl} \sim N(0, \sigma_\varepsilon^2)$ to model unexplained noise in the data. It is assumed that $(\theta_i^{(trial1)}, \theta_i^{(trial4)})$ has a bivariate distribution with mean (0,0) and a covariance matrix that is estimated from the data.

The parameters of the model should be interpreted as follows:

$\mu^{(j)}$= Predicted rating at trial *j* for GS3 (i.e. T=0) for subjects of HC (i.e. $G = 0$), and an average random intercept value (i.e. $\theta_i^{(j)} = 0$).

$\beta_{LIN}^{(j)}$= Average change in the predicted rating for GS *k* at trial *j* compared with GS *k-1* for subjects of HC (i.e. $G = 0$).

$2 * \beta_{QUAD}^{(j)}$ = Expected change in the slope of the linear trend for GS *k* at trial *j* compared with GS *k-1* for subjects of HC (i.e. $G = 0$).

$\beta_G^{(j)}$= Average difference in the predicted rating for GS_3 (i.e. T=0) at trial *j* for FM versus HC.

$\beta_{LINxG}^{(j)}$= Interaction effect between the linear trend and the group variable. This effect represents the difference in the slope of the linear trend component for trial *j* for FM versus HC.

$2 * \beta_{QUADxG}^{(j)}$= Difference in the expected change in the slope of the linear trend for trial *j* for GS *k* compared with GS *k-1* for FM versus HC.

$\sigma^2_{(j)}$ = Variance of the subject-specific predicted ratings at trial *j* for GS3 (i.e. T=0).

σ^2_{ε} = Variance of the error term.