Extinction of Fear Generalization: A Comparison Between Fibromyalgia Patients and Healthy Control Participants

Ann Meulders, *, 1, 3 Michel Meulders, 1, 8 Iris Stouten, *, 1 Jozef De Bie, 4, 11 and Johan W. S. Vlaeyen *, 1, **

*Research Group Health Psychology, 1 Center for Excellence on Generalization Research in Health and Psychopathology, 1 Center for Information Management, Modeling and Simulation, 1 Research Group on Quantitative Psychology and Individual Differences, KU Leuven University, Leuven, Belgium.
2 Multidisciplinary Pain Center, Ziekenhuis Oost-Limburg, Genk, Belgium.
3 Center for Translational Psychological Research (TRACE), Ziekenhuis Oost-Limburg and KU Leuven University, Leuven, Belgium.
4 Department of Clinical Psychological Science, Maastricht University, Maastricht, The Netherlands.

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), 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 control participants (HC). During acquisition, one movement (CS1) predicted pain; another did not (CS0/C0). We tested (extinction of) fear generalization to 5 GSs varying in similarity with the CS1 and CS0/C0. Results revealed flatter pain expectancy generalization gradients in FM than in HC due to elevated responses to GSs more similar to the CS0/C0; the fear generalization gradients did not differ. Although pain-related fear and expectancy to the GSs decreased during extinction, responses 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 nonpainful movement—decrease during extinction in HC and FM. 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.

© 2016 by the American Pain Society

Key words: Pain-related fear, fear conditioning, fear generalization, voluntary movement paradigm, fibromyalgia, learning, fear extinction.

Accumulating evidence indicates that pain-related fear plays a fundamental role in the transition from acute to chronic disabling pain. 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 3 response systems: 1) verbal responding (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. 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 [CRs]), whereas the control stimulus never paired with the pain-US (CS−) 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 (eg, a baby, a shopping bag). One possible mechanism accounting for this spreading of fear is stimulus generalization. Stimulus generalization is also adaptive because 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 generalization stimuli (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 showed that fear indeed spreads selectively toward novel movements resembling the original painful movement in healthy pain-free control participants (HC), but fear generalized in a nondifferential way in fibromyalgia patients (FM). 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 reported that chronic hand pain patients overgeneralized pain-outcome expectancy to novel cues that were more similar to the original ‘safe’ cue compared with HC. 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 more resemble the original CS−, 2) impaired extinction of unreinforced GSs, whereas generalized pain-related fear will subside quickly in HC.

Methods

Participants

This study used a convenience sample of 60 participants including 2 age-matched diagnostic groups: 30 FM (29 female, mean ± SD age = 41 ± 11; range, 22–59 years), and 30 HC (mean ± SD age = 41 ± 12; range, 21–60 years). In line with Meulders et al, we chose not to use absolute age-matched groups, but used 5-year ranges to match the HC to the 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 new diagnostic criteria for fibromyalgia on the basis of the combined Widespread Pain Index (range, 0–19) and Symptom Severity Score (range, 0–12; 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 analphabetsm, pregnancy, current or history of cardiovascular disease, chronic or acute respiratory disease (eg, asthma, bronchitis), neurological diseases (eg, epilepsy), uncorrected hearing problems, having pain at the dominant hand, wrist, or arm that hinders movement of 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, 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, 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 received the book ‘Mastering Your Pain’ [de Pijn van de Baas] by Frits Winter, and HC received a box of Belgian chocolates; both remunerations had an approximate value of €15. As expected, FM had lower educational level, were more likely to be unemployed, and were taking more medication than the HC. More detailed demographic and clinical characteristics can be found in Table 1.
Stimulus Material and Apparatus

The experiment was run on a Windows 7 computer (Dell OptiPlex 7010; Dell Inc., Round Rock, Texas) with 4096MB RAM and an Intel Core i5-3570 CPU processor (Intel Corp., Santa Clara, California) at 3.40 GHz and an AMD Radeon HD 7570 graphics card (Advanced Micro Devices, Sunnyvale, California) 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 Corp, Austin, TX). The conditioned stimuli (CSs) and the GSs were 7 equally spaced movement quadrants, which are part of a semicircle (Fig 1). These proprioceptive stimuli consisted of moving a Paccus Hawk joystick (Paccus Interfaces BV, Almere, The Netherlands) with the dominant hand within 1 of the 7 movement quadrants. Movements in quadrant 1 (ie, 90° to the left) and in quadrant 7 (ie, 90° to the right) served as CSs, and movements in the intermediate (2–6) quadrants, served as GSs. During acquisition, one movement direction (CS1) was followed by the pain-US (75% of the trials), whereas 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, Welwyn Garden City, United Kingdom) and administered through surface SensorMedics electrodes (8 mm; SensorMedics Corp., Yorba Linda, California) filled with K-Y gel (Johnson & Johnson, New Brunswick, New Jersey) 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 = .35, range = 7–8) for the FM group, and 7.98 (SD = .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 eye blink startle response. The eye blink startle response is a component of the reflexive cross-species, full-body defensive response mobilization, which is triggered by startle-evoking stimuli (eg, acoustic startle probe) and can be measured by the tension in the muscles

Table 1. Demographic and Clinical Characteristics for the FM Group (n = 30) and the HC Group (n = 30) Separately

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>FM GROUP</th>
<th>HC GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected pain intensity level, mA</td>
<td>18.73</td>
<td>22.90</td>
</tr>
<tr>
<td>Selected self-reported pain intensity (range 1–10)</td>
<td>7.87 .35</td>
<td>7.98 .18</td>
</tr>
<tr>
<td>Age, y</td>
<td>40.80</td>
<td>40.63</td>
</tr>
<tr>
<td>WPI (range 0–19)</td>
<td>9.43</td>
<td>8.63</td>
</tr>
<tr>
<td>SS (range 0–12)</td>
<td>8.13</td>
<td>1.66</td>
</tr>
<tr>
<td>Highest education level, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Vocational secondary education</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Technical secondary education</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>General secondary education</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Professional bachelor’s degree</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Academic bachelor’s degree</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>Master’s degree</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Type of medication, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Analgesics (opioids)</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Analgesics (nonopioids)</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>47</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: SS, Symptom Severity Score; WPI, Widespread Pain Index.

NOTE. Total N = 60. For WPI, the higher the score, the more pain complaints on different sites of the body during the past week; for SS, 0 = no symptoms, 12 = very much pain symptoms. Other medication includes: muscle relaxants, hormones, antihypertension, antiarrhythmic agents, gastric ulcer medication, dopamine, synthetic thyroid hormone, psoriasis medication, magnesium supplements, and probiotics.
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; Philips, Amsterdam, The Netherlands). Eye blink startle responses elicited by startle probes delivered during the CS/GS movements served as an index of cued pain-related fear. Eye blink startle responses elicited by startle probes during the intertrial interval (ITI) served as an index of contextual pain-related fear.

**Experimental Setting**

Participants were seated in an armchair (.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.

**Procedure**

The experiment was conducted during a 2-hour session and comprised 6 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. In a mixed design (Table 2) participants in both groups (between-subjects factor: FM vs HC) received a pain-US after moving the joystick to the CS\(_1\) direction on 75% of the trials, but never received a pain-US when moving the joystick to the CS\(_1\)/CS\(_0\) direction. Note that the movement direction that served as the CS\(_1\) and CS\(_0\) 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

![Schematic overview of the experimental task during the generalization phase.](image)

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

**Table 2. Study Design Summary**

<table>
<thead>
<tr>
<th>Practice (2 × 8 Trials)</th>
<th>Habituation (8 Trials)</th>
<th>Acquisition (3 × 8 Trials)</th>
<th>Transfer of Acquisition (8 Trials)</th>
<th>Generalization (4 × 7 Trials)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 × [4 × CS+ only]</td>
<td>8 Probes</td>
<td>3 × (4 × CS+)</td>
<td>4 × CS+</td>
<td>4 × CS+</td>
</tr>
<tr>
<td>2 × [4 × CS−]</td>
<td></td>
<td>3 × (4 × CS−)</td>
<td>4 × CS−</td>
<td>4 × GS1−5</td>
</tr>
</tbody>
</table>

NOTE. CS = movement quadrant 1 and 7; GS = 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 nonreinforcement 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.
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 (Gs).

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 eye blink startle responses and the electrocutaneous stimulus were attached (see the Stimulus Material and Apparatus section).

Practice
Fig 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 toward the counter bars, each divided in 4 equal segments, positioned at the end of the 2 equally spaced movement quadrants (1 and 7). Successful movements always resulted in coloring 1 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 (ie, 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, 2 blocks of 8 trials (4 left/4 right) 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.

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 12 seconds, with an ITI of 2 seconds. During each trial, 1 startle probe (100 dBA burst of white noise) was delivered between the eighth 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.

Acquisition
This phase was basically the same as the practice phase (Fig 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 3 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 seconds and a post-CS interval of 8 seconds. The pain-US was presented on 75% of the CS+ trials, but never on CS− trials. In each block of 8 CS movements, 4 of the startle probes were presented during the CS movements, and 4 during the ITI (between 3000 and 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.

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 1 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 seconds followed. During the transfer phase, 1 block of 8 trials (4 left/4 right) was run. The reinforcement scheme and timing scheme of the presentation of the startle probes remained the same as during the acquisition phase.

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 5 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 (ie, quadrants 1–7). One block consists of 7 movements (1 in each movement quadrant); the order of the movements was randomized across participants. As before, after a 3-second 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 to the signaled direction. After successfully performing the movement, again a post-CS/GS ITI of 8 seconds followed. On each trial a startle probe was delivered during the GS/CS movement; no ITI probes were delivered.

Measures

Manipulation Checks

Affective valence, arousal, and control. After the practice, acquisition, and generalization phase, participants completed 3 Self-Assessment Manikin (SAM) scales4 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,” and “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).

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.’

Eye blink startle modulation. Orbicularis oculi electromyographic activity (EMG) was recorded with 3 Ag/AgCl SensorMedics electrodes (4 mm) filled with electrolyte gel. After cleaning the skin with exfoliating peeling cream to reduce interelectrode resistance, electrodes were placed on the left side of the face according to the site specifications proposed by Blumenthal et al.3 The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75-04; Coulbourn Instruments, Whitehall, Pennsylvania). 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.”

Postexperimental 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.

Pain severity. The Chronic Pain Grade Scale (CPGS)53 assesses pain intensity and interference with normal daily activities using 7 items (eg, 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 past 6 months (range, 0–180). On the basis of the pain intensity score, the disability points (on the basis of the disability score and the days of disability) respondents are classified into 4 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.

Pain cognitions. The Pain Cognition List (PCL)49 consists of 39 items divided into 5 subscales (catastrophizing, limitation, optimism, internal control, and trust). Each item presents a specific pain cognition statement (eg, “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; and trust: range, 4–20).

Fear of movement. The Tampa Scale of Kinesiophobia (TSK)72 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 (eg, “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 4-point Likert scale, ranging from 1 “strongly disagree” to 4 “strongly agree” (total score range, 17–68).
Pain disability. The Fibromyalgia Impact Questionnaire (FIQ)\textsuperscript{3} assesses the effect of fibromyalgia on the respondent's daily activities. The FIQ is composed of 10 items. The first item contains 11 questions (eg, “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 to 100, with 0 indicating no impairment at all and 100 maximum impairment.

Affect. The trait version of the Positive and Negative Affect Schedule (PANAS)\textsuperscript{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 (range, 10–50) and 10 items describe negative feelings and assess negative affectivity (range, 10–50).

Depression and anxiety. The Hospital Anxiety Depression Scale (HADS)\textsuperscript{35,60} consists of 14 items divided into 2 subscales (anxiety and depression). Respondents are asked to indicate for each item (eg, “I still enjoy the things I used to enjoy”), which answer reflects best how they felt during past week. Answers are scored from 0 to 3. The scores for the depression subscale and the anxiety subscale range from 0 to 21.

Fear of pain. The Fear of Pain Questionnaire (FPQ)\textsuperscript{28,43} 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 (eg, “Breaking your arm”). Answers are scored from 0 “not fearful” to 5 “extreme fearful.”

Rumination. To explore the way participants typically think about negative experiences and problems, we administered the Perseverative Thinking Questionnaire (PTQ).\textsuperscript{10} This questionnaire comprises 15 items. The item pool included 3 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.”

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>FM GROUP (n = 29)</th>
<th>HC GROUP (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>CPGS, pain intensity*</td>
<td>65.29</td>
<td>14.18</td>
</tr>
<tr>
<td>CPGS, pain disability*</td>
<td>56.90</td>
<td>17.36</td>
</tr>
<tr>
<td>CPGS, number of days disability*</td>
<td>44.90</td>
<td>66.47</td>
</tr>
<tr>
<td>PCL, catastrophizing*</td>
<td>44.17</td>
<td>13.36</td>
</tr>
<tr>
<td>PCL, limitation*</td>
<td>27.14</td>
<td>5.12</td>
</tr>
<tr>
<td>PCL, optimism</td>
<td>23.86</td>
<td>4.31</td>
</tr>
<tr>
<td>PCL, internal control</td>
<td>16.1</td>
<td>4.18</td>
</tr>
<tr>
<td>PCL, trust</td>
<td>13.41</td>
<td>2.82</td>
</tr>
<tr>
<td>TSK, total score*</td>
<td>41.07</td>
<td>7.93</td>
</tr>
<tr>
<td>FIQ, total score*</td>
<td>55.35</td>
<td>15.15</td>
</tr>
<tr>
<td>PANAS, positive affect*</td>
<td>30.45</td>
<td>7.87</td>
</tr>
<tr>
<td>PANAS, negative affect*</td>
<td>24.14</td>
<td>8.53</td>
</tr>
<tr>
<td>HADS, anxiety*</td>
<td>17.72</td>
<td>3.24</td>
</tr>
<tr>
<td>HADS, depression</td>
<td>15.45</td>
<td>2.23</td>
</tr>
<tr>
<td>FPQ, medical pain</td>
<td>18.90</td>
<td>6.76</td>
</tr>
<tr>
<td>FPQ, minor pain</td>
<td>16.72</td>
<td>4.98</td>
</tr>
<tr>
<td>FPQ, severe pain</td>
<td>24.14</td>
<td>8.36</td>
</tr>
<tr>
<td>FPQ, total score*</td>
<td>59.76</td>
<td>16.30</td>
</tr>
<tr>
<td>PTQ, total score*</td>
<td>27.76</td>
<td>13.79</td>
</tr>
</tbody>
</table>

NOTE. Total sample N = 59. One patient failed to fill out the questionnaires. CPGS subscales are calculated for pain intensity (items 1–3), pain disability (items 4–6) and days of disability (item 7); PCL subscales are calculated for catastrophizing, limitation, optimism, internal control, and trust; PANAS subscales are calculated for positive affect and negative affect; HADS subscales are calculated for anxiety and depression; FPQ subscales are calculated for medical pain, minor pain, and severe pain. On the basis of the CPGS scales, 10% (3 of 29) of the FM were classified as grade I (low disability, low intensity), 28% (8 of 29) as grade II (low disability, high intensity), 14% (4 of 29) as grade III (high disability, moderately limiting), and 48% (14 of 29) as grade IV (high disability, severely limiting).

\*P < .05, after Holm-Bonferroni corrections.

Response Definition and Data Analysis

Overview

Response Definition of the Startle Modulation

Using PSPHA (Psychophysiological Analysis; PSPHA v21/2/2011),\textsuperscript{8} a modular script-based program, we calculated the peak amplitudes defined as the maximum of the response curve within 21 to 175 ms after the startle probe.
onset. All startle waveforms were visually inspected offline, 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 to 20 ms time window after probe onset, also when no visual peak could be detected (ie, nonresponse), the response was rejected. Participants who failed to reach elevated peak amplitudes compared with baseline on more than 50% of the trials were considered nonresponders 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 eye blink 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 interindividual 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 illustrations, 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.

Data Analysis Overview

Some preparatory analyses were necessary to test our main research questions: First, as a manipulation check, we carried out a series of repeated measures analyses of variance (RM 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 η² is reported for all omnibus ANOVA effects, and Greenhouse-Geisser corrections were applied when appropriate (uncorrected degrees of freedom and corrected P values are reported together with η²). 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 compared with the beginning. Therefore, we defined a random intercept 2-level linear regression model to analyze the test effects of stimulus type (CS+/CS−) on the change in average pain-related fear ratings during 4 repeated measurements (ie, during the 3 blocks of acquisition [A1/A2/A3] and 1 block of the transfer of acquisition phase [T1]), nested within persons for both groups (FM and HC; see the Supplementary Text for the detailed statistical model description). The effects included in this model were estimated simultaneously using the SAS procedure MIXED. Both models are able to predict participants’ pain-US expectancy and fear of movement-related pain (see the Supplementary Material text for the detailed statistical model description). The effects included in this model were estimated simultaneously using the SAS procedure MIXED.44,50 Both models and expected that: 1) HC would show an immediate transfer of differential pain-related fear and expectancy learning, whereas FM would show an initial loss of differential learning due to increased CS− responding, 2) FM 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. To test the first hypothesis, we defined two 2-level linear regression models (1 for each dependent variable) with 2 random intercepts (1 for each stimulus type) including group (FM and HC), linear trend variable Tj (which equals 0, 1, 2, and 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 the Supplementary Table 2) very well because they explain 84% and 83%, respectively, of the observed variance in ratings. Including random intercepts was necessary because 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.

To test the second and third hypothesis, we defined two 2-level linear regression models (1 for pain-US expectancy and 1 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 Tj (which equals −3, −2, −1, 0, 1, 2, and 3 for stimulus k = CS+, GS1, GS2, GS3, GS4, GS5, GS6, and CS−) modeling the linear component of the generalization gradient together with a quadratic trend variable (Tj²) modeling the quadratic component of the generalization gradient, as well as the interaction terms between group and the linear (quadratic) trend variable (see the Supplementary Material text for the detailed statistical model description). The inclusion of the quadratic trend is supported by the data because the models including a quadratic trend have a better balance between complexity and goodness of fit (ie, they have lower values of the Bayesian information criterion). Bayesian information criterion values for the model including only
the linear trend were higher than for the model including the linear as well as the quadratic trend, for both dependent variables, respectively pain-US expectancy: 3,914 versus 3,874, and fear of movement-related pain: 3,414 versus 3,396. The effects included in this model were estimated simultaneously using the SAS procedure MIXED.44,50 Both models are able to predict participants’ pain-US expectancy and fear of movement-related pain ratings (Supplementary Table 3) quite well because they explain 62% and 65%, respectively, of the observed variance in ratings. Including random intercepts was necessary because 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 eye blink measures for either of the groups; so as not to overload the results section these analyses are omitted. Finally, to test our fourth hypothesis, pain-US intensity and unpleasantness ratings were analyzed using RM 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 the multilevel modeling approach as well as the standard mixed RM ANOVA because unlike standard mixed RM ANOVA the multilevel approach: 1) can model a quadratic trend, 2) supports the estimation of a large variety of planned contrasts (ie, all contrasts can be expressed as a linear function of the underlying regression parameters), and 3) supports the inclusion of random coefficients (eg, 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 acquisition in the startle measures, as well as to test hypothesis 4, a more standard mixed RM ANOVA was satisfactory.

Results

Pain-US Characteristics and Questionnaires

In contrast with Meulders et al,10 FM did not select a lower 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 (Table 1). For the analyses of the questionnaires, 1 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-Bonferroni14 correction is based on the ranking of the P values of one family of hypotheses from smallest to largest. ‘m’ is the number of P values. If the first P value is ≥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 with α/(m−1), etc. Corrected P values are reported. As expected, the groups had significantly different scores on most of the psychological trait questionnaires (Table 3). Compared with the HC group, FM 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 past 6 months, t_{57} = 3.61, P < .01 (CPGS). 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 compared 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 compared with the HC group, t_{57} = −.24, P = .81 (FPQ). Furthermore, no differences were found with respect to the FPQ subscales, medical pain, t_{57} = −.86, P = .79, and severe pain, t_{57} = −1.15, P = 1.00, but FM tended to have higher scores for the minor pain subscale, t_{57} = 2.82, P = .05. FM also reported more repetitive negative thinking, t_{57} = 4.09, P < .0001, than the HC group (PTQ).

Manipulation Checks

For all 3 SAM ratings, we performed a 2 (group: FM/HC) × 2 (stimulus type: CS+/CS−) × 3 (phase: practice/acquisition/generalization) mixed RM ANOVA.

Affective Valence of the CS Movements

This analysis revealed significant main effects for group, F_{1,58} = 48.00, P < .05, η^2_p = .03, stimulus type, F_{1,58} = 37.19, P < .001, η^2_p = .13, and phase, F_{2,116} = 5.99, P < .01, η^2_p = .04. Further, there was a significant Stimulus Type × Phase interaction, F_{2,116} = 26.96, P < .0001, η^2_p = .32, 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 regard to the affective valence of the painful
CS+ movement, $F_{1,58} = 1.08$, $P = .30$, but FM were more unhappy when performing the safe CS− movement, $F_{1,58} = 7.45$, $P < .01$.

### Arousal Elicited by the CS Movements

This analysis showed a significant main effect for stimulus type, $F_{1,58} = 24.12$, $P < .001$, $\eta^2_G = .05$. Further there was a significant Stimulus Type × Phase interaction, $F_{2,116} = 6.52$, $P < .01$, $\varepsilon = .78$, $\eta^2_C = .02$, 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 × Group interaction was significant, $F_{2,116} = 5.69$, $P < .01$, $\varepsilon = .84$, $\eta^2_C = .03$, 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, HC: $F < 1$; FM: $F_{1,58} = 3.99$, $P = .051$. 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: $F_{1,58} = 8.99$, $P < .01$.

### Feelings of Being in Control When Performing CS Movements

This analysis yielded significant main effects for stimulus type, $F_{1,58} = 23.48$, $P < .001$, $\eta^2_G = .05$, and phase, $F_{2,116} = 10.21$, $P < .001$, $\varepsilon = .80$, $\eta^2_C = .05$. Further there was a significant Stimulus Type × Phase interaction, $F_{2,116} = 10.19$, $P < .001$, $\varepsilon = .86$, $\eta^2_C = .05$, 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 × Group interaction also reached significance, $F_{2,116} = 5.81$, $P < .01$, $\varepsilon = .80$, $\eta^2_C = .03$, 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: $F_{1,58} = 12.74$, $P < .001$.

### 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 the Supplementary Material for the detailed statistical model description). Supplementary Table 1 presents the results of the multilevel regression model for fear of movement-related pain ratings during acquisition (A1–3) and transfer of acquisition (T) for both groups (HC and 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− (Fig 2). This was indicated by a significant difference in slopes for the CS+ and the CS− in both groups ($\beta_{S_{HC}}^{A1–3} - \beta_{S_{FM}}^{A1–3} = .93$, $P < .0001$; $\beta_{S_{HC}}^{A1–3} - \beta_{S_{FM}}^{A1–3} = .55$, $P < .05$). Differential fear of movement-related pain was already acquired at the first rating moment A1 (that is, after 4
movements of each type) in the HC, that is, they reported higher fear in response to the CS+ than the CS−/C0 (g1 = 1.26, P < .01), whereas this was not the case for the FM (g2 = .76, P = .06). At the last rating moment T (after the transfer of acquisition phase) the fear elicited by the CS+ was significantly greater compared with the CS− movement in both groups (g3 = 2.90, P < .0001; g4 = 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.

Acquisition of Fear-Potentiated Eye Blink Startle

A 2 (group: FM/HC) × 3 (stimulus type: CS+/CS−/ITI) × 4 (block: A1–A3, T) mixed RM ANOVA was carried out to test acquisition of differential fear learning in the eye blink startle measures (Fig 3). The results showed a significant main effect for block, F3,159 = 3.03, P < .05, ε = .92, ηp2 = .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, F2,106 = 4.11, P < .05, ε = .94, ηp2 = .02. The Block × Stimulus Type interaction however, just failed to reach significance, F6,318 = 2.16, P = .05, ε = .89, ηp2 = .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 eye blink amplitudes were elevated during the CS+ movement compared with the CS− movement, F1,53 = 10.95, P < .01. These data confirm that participants in both groups successfully acquired similar levels of differential eye blink startle responding.

Testing Our Primary Hypotheses

Hypothesis 1: Differences in Transfer of Acquisition Between HC and FM for Pain-US Expectancy and Fear of Movement-Related Pain Ratings

Supplementary Table 2 presents the results of the multilevel regression model for the pain-US expectancy and fear of movement-related pain ratings during the 4 trials of the transfer of acquisition phase; follow-up planned contrasts are depicted in Fig 4. When reporting planned contrasts, $\hat{Y}_j^i (I)$ 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; Fig 4A) HC still expected the pain-US to occur more after the $CS^+$ than after the $CS^-$ ($\gamma_{15} = 3.37, P < .0001$). 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 (Fig 4B).

Similarly, for the fear of movement-related pain ratings, at $T1$ (the first trial of the transfer of acquisition phase; Fig 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 $T1$ ($\gamma_{18} = .88, P = .09$). As expected, this lack of differentiation was due to elevated fear responses for the $CS^-$ in the FM 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: $\gamma_{28} = 2.76, P < .0001$; Fig 4D).

**Hypothesis 2: Differences in Generalization Between HC and FM**

Supplementary Table 3 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 shown in Fig 5. Note that when reporting contrasts $\gamma_j^k$ ($l$) represents the predicted rating for stimulus $k$ ($CS^+$, $GS1$, $GS2$, $GS3$, $GS4$, $GS5$, $CS^-$) at trial...
(T1,T4) in group (HC, FM). The generalization effect is
expected to be the largest at T1, and to extinguish in
the following trials, therefore the difference in
generalization between the HC and FM is assessed at T1 and
differences in extinction of generalization are assessed at T4
(and differences from T1 to T4). 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, $p_{LINCG}^{111} = .25, P < .05$, indicating that FM showed
flatter pain-US expectancy generalization gradients
compared with the HC. Planned comparisons (Fig 5A)
further confirmed that this difference in steepness of the
slopes is explained by differences at the CS− side of
the generalization gradient (ie, responses to novel move-
ments that are more similar to the safe movements), but
that no differences occurred at the CS+ side of the
generalization gradient (ie, responses to novel movements
that are more similar to the painful movements). More parti-
cularly, FM reported significantly higher pain-US expect-
cations for the CS− ($\gamma_{23} = 2.23, P < .01$), and G55 (the
generalization movement that was most similar to the
CS−), $\gamma_{39} = 1.46, P < .05$, but the HC; the pain-US expect-
cancy 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, $p_{LINCG}^{111} = -.02, P = .78$, sug-
gestig 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, $p_{LINCG}^{111} = .08, P = .08$. Planned contrasts (Fig 5B) further showed that FM
were more afraid of the original CS− ($\gamma_{36} = 1.27, P < .05$), but they also reported more fear of
movement-related pain in response to the original CS−
($\gamma_{37} = 1.40, P < .05$); these elevated fear responses seemed to spread on both sides of the generalization gradient, but failed to reach significance (G51: $\gamma_{38} = 1.00, P = .07,$
and G55: $\gamma_{39} = .91, P = .09$). The fear of movement-
related pain ratings for the other GSs did not differ be-
tween both groups ($\gamma_{40}, \gamma_{41}, \gamma_{42}$).

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 con-
tinuum in FM.

Hypothesis 3: Differences in Extinction of
Generalization Between HC and FM

Predicted ratings of pain-US expectancy and fear of
movement-related fear are depicted in Fig 5. At T4, there
was a significantly different slope in the pain-US expec-
tancy ratings for the FM versus HC, $p_{LINCG}^{111} = .22, P < .05$.

Planned contrasts (Fig 5C) further showed that pain-US
expectancies in response to all GSs decreased signiﬁcantly
from T1 to T4 for the HC ($\gamma_{43} = 1.47, \gamma_{44} = 2.41,$
$\gamma_{45} = 2.77, \gamma_{46} = .35, \gamma_{47} = 1.71; all Ps < .0001$), but not
for the CS− ($\gamma_{48} = -.06, P = .91$), that remained rein-
fforced during the generalization phase, and not for
the CS− ($\gamma_{49} = .30, P = .56$; ie, floor effect). The pain-US
expectancy ratings for the FM also declined signiﬁcantly
from T1 to T4 for GS2 ($\gamma_{50} = 1.19$), G53 ($\gamma_{51} = 1.42$), G54
($\gamma_{52} = 1.36$), and G55 ($\gamma_{53} = 1.03$; all Ps < .01) but not for
the CS− ($\gamma_{54} = -.10, P = .84$), the CS− ($\gamma_{55} = .42,
$P = .42$), and the GS1 ($\gamma_{56} = .68, P = .05$). Interestingly,
the decline in pain-US expectancies for GS2 ($\gamma_{56} = \gamma_{57} = – 1.22$), G53 ($\gamma_{58} – \gamma_{44} = – 1.35$), and G54
($\gamma_{52} – \gamma_{45} = – 1.17; all Ps < .05$), 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
T4, the pain-US expectancies for all GSs (G51: $\gamma_{51} = 1.26$,
$P < .05$; GS2: $\gamma_{52} = 1.64, P < .01$; GS3: $\gamma_{53} = 1.91,
$P < .001$; GS4: $\gamma_{54} = 2.08, P < .001$; G55: $\gamma_{55} = 2.18,\ $
P < .0001), and the CS− ($\gamma_{57} = 2.11, P < .01$), were indeed
still signiﬁcantly higher for the FM than the HC, which
further supports the resistance to extinction of general-
ation hypothesis (Fig 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 rat-
ings. At T4, there was no signiﬁcantly different slope in
fear of movement-related pain ratings for the FM versus
HC ($p_{LINCG}^{111} = -.05, P = .51$). Planned contrasts (Fig 5D)
further showed that fear in response to all GSs decreased
signiﬁcantly from T1 to T4 for the HC (G51: $\gamma_{59} = .66,$
$P < .05$; GS2: $\gamma_{60} = .98, P < .001$; GS3: $\gamma_{61} = 1.09,$
$P < .0001$; GS4: $\gamma_{62} = 2.08, P < .001$; G55: $\gamma_{63} = .70,$
$P < .01$), but not for the CS− ($\gamma_{64} = .15, P = .69$), that re-
mained reinforced during the generalization phase, and
not for the CS− ($\gamma_{65} = .22, P = .57$; ie, floor effect). The
fear of movement-related pain ratings for the FM also
declin ed signiﬁcantly from T1 to T4 for GS2 ($\gamma_{66} = .73,$
$P < .01$), G53 ($\gamma_{67} = .86, P < .001$), GS4 ($\gamma_{68} = .81, P < .01$),
and G55 ($\gamma_{69} = .59, P < .05$), but not for the CS− ($\gamma_{70} = -.06, P = .87$), the CS− ($\gamma_{71} = .18, P = .63$), and
the GS1 ($\gamma_{72} = .42, P = .10$). The decline in fear of
movement-related pain was not signiﬁcantly smaller in
the FM group than the HC (Fig 5D). At T4, however the
fear of most generalization movements was still signiﬁ-
cantly higher for the FM than the HC (G51: $\gamma_{73} = 1.24,$
$P < .01$; GS2: $\gamma_{74} = .99, P < .05$; GS3: $\gamma_{75} = .87, P = .06$;
GS4: $\gamma_{76} = .89, P = .05$; G55: $\gamma_{77} = 1.03, P < .05$), which at
least provides partial support for the resistance to extinc-
tion of generalization hypothesis (Fig 5C).

Hypothesis 4: Differences in Pain-US
Intensity and Unpleasantness Between
HC and FM

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, T, generalization). These analyses yielded
significant main effect of group (unpleasantness: $F_{1,58} = 4.91, P < .05, \eta^2_p = .10$; intensity: $F_{1,58} = 8.26, P < .05, \eta^2_p = .06$). 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.

Discussion

To our knowledge, this is the first study that investigated the differences in generalization gradients of pain-related fear and expectancy between FM and age- and gender-matched 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 with 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, and 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 with the CS– movement. Interestingly, whereas HC acquired these CS-US contingencies after only 1 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 setup, 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 onward, but FM did not. These results seem to suggest that when 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 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–no US association that inhibits the behavioral expression of the first learned association rather than the forgetting/overwriting of the original CS–US association. As a consequence, which of both coexisting associations controls behavior is context-dependent. The effect observed in our study is very similar to renewal (ie, a return of fear after successful extinction due to a context switch) because the acquired safety learning disappears when a context change occurs (ie, signaled vs voluntary movement setup). These findings corroborate previous findings of Meulders et al, and suggest that FM, who seem to be characterized by fragile safety learning, might have difficulties transferring CS–no US 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. 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 judgements in chronic hand pain patients. 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 4 unreinforced trials, pain expectancies for all generalization movements remained elevated for FM compared with 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 4 unreinforced trials, pain-related fear of most of the generalization movements remained elevated for the FM compared with the HC. These results suggest a deficiency in the extinction of fear generalization in FM, which may contribute to the maintenance of chronic disability in patients. Closely related, Flor and colleagues previously showed that, relative to HC, chronic back pain patients showed similar rates of acquisition, but slower extinction of verbal as well as cortical pain responses. The current findings are also in line with previous research on fear extinction in anxiety disorder patients. For example, Wessa and Flor reported that post-traumatic stress disorder patients have a deficit in extinction of traumatic response. Michael et al conducted a study with panic disorder patients and reported that panic disorder patients showed impaired extinction learning. The current findings corroborate previous research in anxiety disorders that reported differences in fear extinction between HC and, 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. 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.

There are some limitations that should be addressed as well. First, 5 participants were excluded from the startle analyses. Because of reduced statistical power, general interactions might have failed to reach statistical significance. Second, only 1 male participated in our study, so the results cannot necessarily be generalized to a male population. However, women are affected with fibromyalgia approximately 3 times more often than men. 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 fibromyalgia. Fourth, the groups also differed with respect to medication use and comorbidity with anxiety and depression, thus we cannot exclude the possibility that this might have contributed to the observed differences in fear learning expression. Indeed, impaired safety learning, and resistance to extinction has been reported in anxiety disorder patients as well. Moreover, anxiolytics might affect the expression of context conditioning. Opioids have been shown to impair fear learning, and antidepressants may enhance cue-d fear conditioning. However, because of the possible opposite effects of the different drugs used in our patient group, it is rather unlikely that the medication use explains all of the observed variance between FM and HC.

Fear generalization research explains how stimuli that were never associated with pain 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, 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 responsibility. 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).

Conclusions
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 because of 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 compared with 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.

Acknowledgments
Part of these data were presented in the symposium on “Protective responses in pain: the role of threat, associative learning, and perception” at the 27th Annual Convention of the Association for Psychological Science, New York, New York, May 2015, and in the “EFIC Symposium: New Findings in Clinical Pain Research: Results of the EFIC-GRÜNENTHAL Grants 2012” at the Ninth EFIC Congress, Pain in Europe IX, Vienna, Austria, September 2015. The authors thank the Centre for Translational Psychological Research (TRACE) for their assistance in the recruitment of the FM.

Supplementary Data
Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jpain.2016.10.004.

References
94 The Journal of Pain


