



Generalization of Conscious Fear Is Positively Correlated with Anxiety, but Not with Depression

Doyoung Park^{1†}, Hwa-Jin Lee^{1†} and Sue-Hyun Lee^{1,2*}

¹Department of Bio and Brain Engineering, College of Engineering, ²Program of Brain and Cognitive Engineering, College of Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea

Generalization of learned fear has been considered to be critical for our survival. Patients with anxiety problems show overgeneralization of learned fear, as reflected by defensive physiological responses to harmless stimuli. Together with these physiological responses, conscious feeling of fear is a seminal part of emotional process that is directly related to the suffering of anxiety patients. However, the effect of anxiety on the generalization of conscious feeling remains unclear. We thus focused on the question whether the generalization of conscious feeling of fear depends on individual anxiety level in nonpatient participants. To address this question, we developed a fear generalization paradigm using natural scene images. We found that subjective feeling of fear was generalized to similar stimuli with the conditioned stimuli (CS), and that this generalization of conscious fear was positively correlated with the level of individual anxiety. Anxiety and depression frequently co-occur, but the individual depression level was not correlated with the fear generalization. These suggest that individual anxiety level mainly affects the generalization of conscious fear.

Key words: generalization, anxiety, conditioning, fear, depression, conscious feeling

INTRODUCTION

Generalization allows humans to extend their previous experiences to novel situations on the basis of the similarity with the experiences [1]. Especially generalization of learned fear has been considered to be critical for our survival [2, 3]. By generalizing past fearful experiences, we can generate appropriate defensive responses to potentially dangerous contexts [1, 2, 4].

Abnormal fear generalization has been reported in patients with anxiety disorders [5-7]. They showed irrational defensive or fear

responses to not only fearful stimuli but also harmless stimuli that bear little similarity with the fearful stimuli. To examine fear responses to the learned threat in anxiety patients, researchers have used fear conditioning paradigm [8, 9]. In a typical fear conditioning, a neutral stimulus (conditioned stimulus (CS+)) predicts the occurrence of an aversive event (unconditioned stimulus (US)), whereas another neutral stimulus (CS-) is paired with the non-occurrence of the aversive event. Compared to healthy participants, the anxiety disordered patients displayed significantly reduced discrimination between CS+ and CS- in physiological fear responses such as skin conductance response (SCR) [10-13]. These results suggest the possibility that the patients with anxiety problems show overgeneralization of learned fear. To investigate this possibility directly, a systematic paradigm of fear generalization was developed by modification of the typical fear conditioning [1, 7, 14]. Lissek et al. [7, 14], used rings of gradually increasing size with

Received December 23, 2017, Revised January 24, 2018,
Accepted January 26, 2018

*To whom correspondence should be addressed.

TEL: 82-42-350-4311, FAX: 82-42-350-4310

e-mail: suelee@kaist.ac.kr

†These authors contributed equally.

extremes serving as CS+ and CS-, and the intermediary size rings serving as generalization stimuli (GS). Consistent with the result of reduced discrimination between CS+ and CS-, panic patients and generalized anxiety disorder (GAD) patients showed fear response to significantly broader range of rings than healthy participants, as reflected by startle electromyography (EMG) [7, 15]. These studies suggest that overgeneralization of conditioned fear is a pathogenic

marker of anxiety disorder, and understanding fear overgeneralization process is critical for the treatments of anxiety disorders [7, 15].

Emotional process involves both conscious feeling and change of physiological responses [16]. Although fear conditioning studies including fear generalization have focused predominately on defensive physiological responses, which were assessed as fear, “fear”

A



B

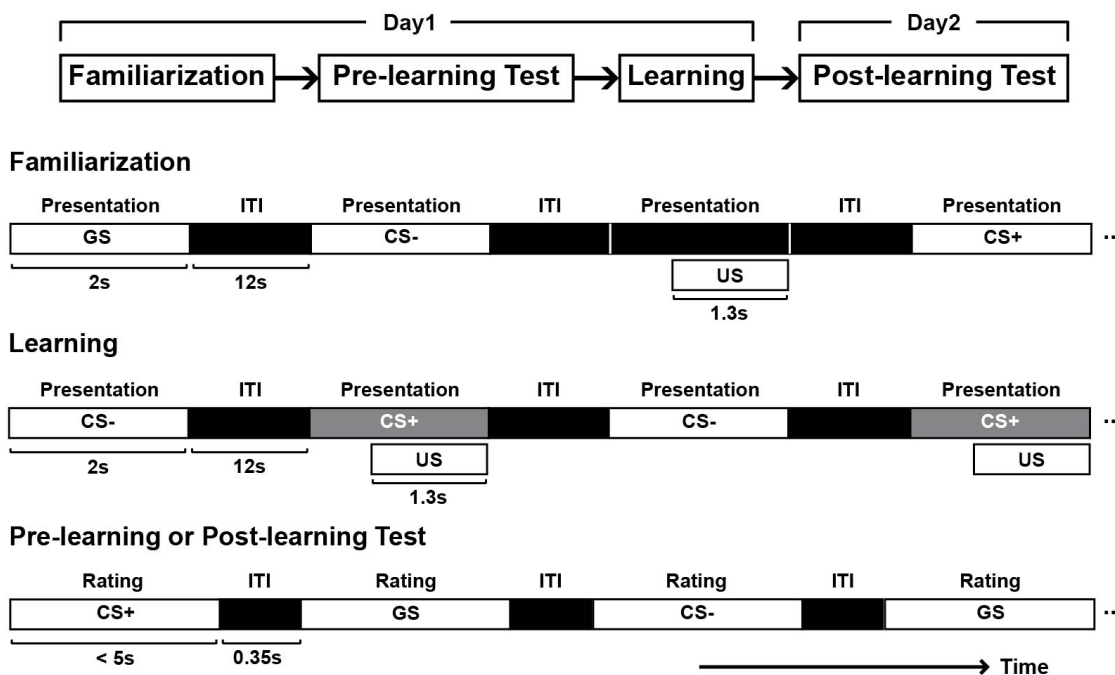


Fig. 1. Experimental design. (A) Sample scene images used in the experiment. Natural scene images from continuously changing seven categories served as conditioned stimuli (CS+), unpaired conditioned stimuli (CS-), and generalization stimuli (GSs). The seven categories were sandy desert, gravel desert, vegetated desert, grassland, mountain, forest, and swamp. Participants were counterbalanced into two groups. For half of the participants (Group 1), scenes of the sandy desert category were paired with US, while scenes of the swamp category were paired with US for the other half (Group 2). (B) The experiment was conducted for two consecutive days. On the first day (Day 1), participants were introduced to the familiarization, pre-learning test, and learning sessions, and they performed post-learning test on the next day (Day 2). During the familiarization, scene images of all seven categories and US were presented in random order. During the learning, only scenes from the CS+ and CS- categories were presented, and half of the CS+ presentations were co-terminated with US. During the pre-learning or post-learning test, participants rated the subjective feeling or US-expectancy for each scene on a 1~5 scale while each scene image was presented. ITI, intertrial interval.

usually refers to the subjective awareness of being afraid (conscious feeling of fear) in our daily life. Moreover, clinical studies have reported that anxiety disordered patients are suffering from the subjective feeling of heightened fearfulness [17]. Thus, not only physiological responses but also conscious feeling of fear should be taken into account in order to fully understand fear overgeneralization observed in the anxiety disordered patients. However, it has not been directly validated whether conscious feeling of fear can be generalized to similar contexts and whether this generalization depends on anxiety level. Although some emotional theories suggest that the conscious feeling is sequentially linked to the physiological responses [16, 18], others argue that the conscious feeling and the physiological response are parallel [16, 19], which implies the physiological responses are not directly responsible for the conscious feeling and vice versa. Further, both theories do not clearly support that the extent to which the conscious feeling changes depends on the change in the physiological response. In addition to the emotional process, the fear generalization studies also measured the explicit knowledge of the relationship between CS+ and US [7, 15] (Emotional process is not always consistent with the explicit knowledge about the emotional event. For example, sometimes we feel fear in the dark room even when we know that nothing is going to happen). While Lissek et al. [7, 15] showed overgeneralization of US expectancy in panic or GAD patients, overgeneralization of US expectancy was not observed in other studies with GAD patients [20, 21]. Thus, the effect of anxiety on the generalization of the explicit knowledge is controversial.

To directly investigate the effect of anxiety on the generalization of conscious feeling of fear, we developed a fear generalization paradigm based on natural scene image stimuli, which are a simplified version of the real complex contexts (Fig. 1). The scene image stimuli consisted of images from continuously changing seven categories (Fig. 1A). The scene images from one extreme category served as conditioned stimuli (CS+), the images from the other extreme category were safety stimuli (CS-), and the images from intermediate categories were used as generalization stimuli (GSs). Here we focused on the effect of individual anxiety level in nonpatient participants, and assessed the relationship between subjective rating of conscious fear and individual anxiety level.

We find that conscious feeling of fear transfers to the GSs that are close to the CS+, and progressively decreases as the tested stimulus becomes less similar to the CS+. This generalization of conscious fear shows significantly positive correlation with the individual anxiety states. In addition, although anxiety and depression often occur together [22, 23], the level of individual depression is more related to the strength of conditioned fear for CS+, but not to fear generalization.

MATERIALS AND METHODS

Participants

21 participants (11 females) with a mean age of 23.29 (SEM=0.80) participated in the experiment. All of them were native Korean speakers and right-handed. All participants provided written informed consent for the procedure in accordance with protocols approved by the KAIST Institutional Review Board.

Stimuli

For a systematic fear generalization system, we modified the typical fear conditioning paradigm. In this system, aversive female scream sound was used as unconditioned stimuli (US). To minimize decrease of fear response due to adaptation induced when the same US is repeatedly presented, 24 different scream sounds were used across the trials of fear conditioning (learning). One scream sound was used per each trial, and the order of the scream sounds were randomized and counterbalanced across the trials. The length of each scream sound was 1.3 s, and the sound stimuli were delivered via a headset with a volume of 100 dB.

Natural scenes images from continuously changing seven categories served as conditioned stimuli (CS+), unpaired conditioned stimuli (CS-), and generalization stimuli (GSs) (Fig. 1A). The seven categories were sandy desert, gravel desert, vegetated desert, grassland, mountain, forest, and swamp. Thus, the scenes were categorized based on the amount of water and plants. Sandy desert is a representative area with little water or plant, and swamp is a typical area with lots of water and plants. We used 3 different scene images per category during familiarization, pre-learning test, or post-learning test. During the learning session, to induce learning that the US follows the context of sandy desert or swamp rather than a particular image, we used four different angle images of three scenes per category (total 12 images of sandy desert and 12 images of swamp). The scene images were selected and modified into grayscale from SUN397 Scene Database [24], searched images on Google (https://commons.wikimedia.org/wiki/File:Sheffield_Park_Panorama.jpg), and panorama images from Google Maps Street View (© 2017 Google). Throughout the experiment, the scene images (8×8°) were viewed via a monitor (1024×768 resolution, 60 Hz refresh rate).

Experimental procedure

The experiment was conducted for two consecutive days in a sound attenuated room where participants completed each of the learning and test sessions individually (Fig. 1B). On the first day (Day 1), participants were introduced to the familiarization, pre-learning test, and learning sessions, and they performed post-

learning test on the next day (Day 2) (Fig. 1B).

During the familiarization session, 21 scene images of all seven categories (three scenes per each category) and two scream sounds that were different from US sounds were presented in random order. During this session, each scene or each scream sound occurred in 2 trials, for a total of 46 trials. On each trial, participants saw 2 s presentation of a scene image or heard a 1.3 s long scream sound. The inter-trial interval was 12 s.

During the pre-learning and post-learning tests, participants first

rated the expectancy that the US would follow each scene (US-expectancy) on a 1~5 scale (1, very likely; 2, likely; 3, not sure; 4, unlikely; 5, very unlikely). Next, they also rated their subjective feeling to each scene from the seven categories on a 1~5 scale (1, very unpleasant; 2, unpleasant; 3, moderate; 4, pleasant; 5, very pleasant). In both ratings, they were instructed to press 1, 2, 3, 4, or 5 within 5 seconds with their right hand while each scene image was presented (Fig. 2). The inter-trial interval was 350 ms.

The learning session was presented in 2 runs consisting of 48 tri-

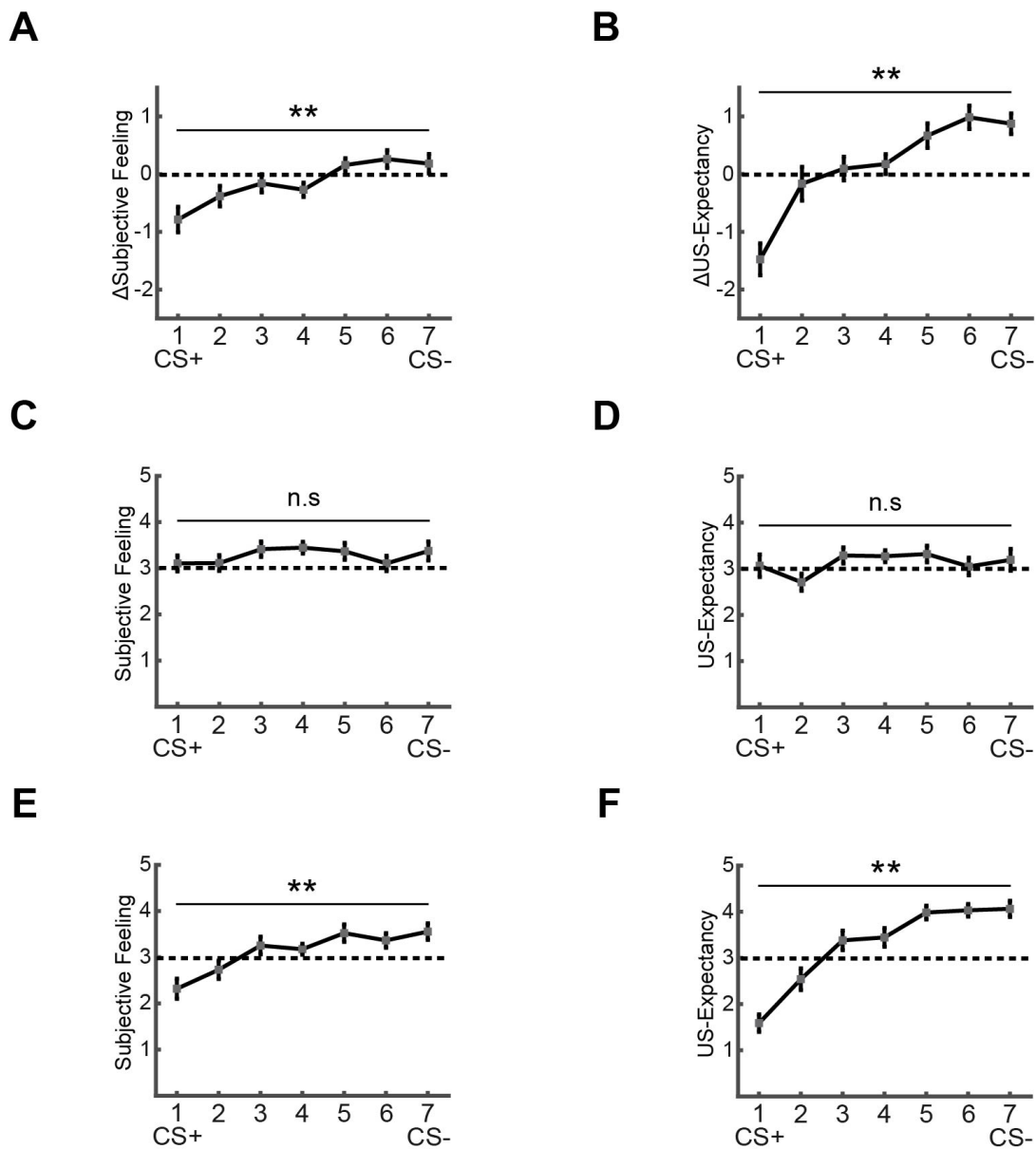


Fig. 2. Generalization of conditioned fear. (A) The change of subjective feeling induced by fear conditioning. (B) The change of US-expectancy. (C) Average subjective feeling scores during the pre-learning test. (D) Average US-expectancy scores during the pre-learning test. (E) Average subjective feeling scores during the post-learning test. (F) Average US-expectancy scores during the post-learning test. Numbers in x-axis indicate scene categories. ** $p < 0.01$, Friedman test for the scene category effect. Error bars indicated between-subjects s.e.m.

als. On each trial, a scene image from the sandy desert or swamp category was presented for 2 s with a 12 s inter-trial interval. For half of the participants (Group 1), scenes of the sandy desert category were paired with US, while scenes of the swamp category were paired with US for the other half (Group 2) (Fig. 1A). Thus, for the participants of Group 1, scenes of the sandy desert category were CS+ and scenes of the swamp category were CS-, while scenes of the swamp category were CS+ and scenes of the sandy desert category were CS- for Group 2 participants. Category 1 indicates CS+ scene category, and category 7 indicates CS- scene category. Scenes from the category 2, 3, 4, 5, or 6, which served as GSs, were used in the familiarization, pre-learning test, and post-learning test sessions, but not during the learning session. Half of the CS+ presentations were co-terminated with 1.3 s long US (aversive female scream sound).

Depression and anxiety level

Before beginning the experimental sessions, participants completed K-BAI (Korean version of Beck Anxiety Inventory) and K-BDI-II (Korean version of Beck Depression Inventory) questionnaires (www.koreapsy.co.kr). BDI and BAI are self-report

inventory created by Aaron T. Beck for measuring the severity of depression and anxiety [25, 26]. Each consists of 21 questions of multiple choice. BDI scores in range 0~9 indicates minimal depression, 10~18 indicates mild depression, 19~29 indicates moderate depression, and 30~63 indicates severe depression. BAI scores in range 0~9 means minimal anxiety, 10~16 means mild anxiety, 17~29 means moderate anxiety and 30~63 means severe anxiety.

Fear generalization analyses

To measure the degree of fear generalization, we derived the maximum category number of scenes for which the participants rated as score 2 ('unpleasant'). In the case when score 2 was never rated throughout the post-learning test session, the maximum category number of scenes rated as score 1 ('very unpleasant') was derived. We defined this maximum category as category threshold. Among 21 participants, 6 participants rated feeling score above 2 for all scenes. So, for the analysis of the category threshold for the subjective feeling, we focused on the scores from the other 15 participants (Fig. 3A and D). We also derived category threshold for US-expectancy. Because 1 participant rated US-expectancy score above 2 for all scenes during the post-learning test, we used

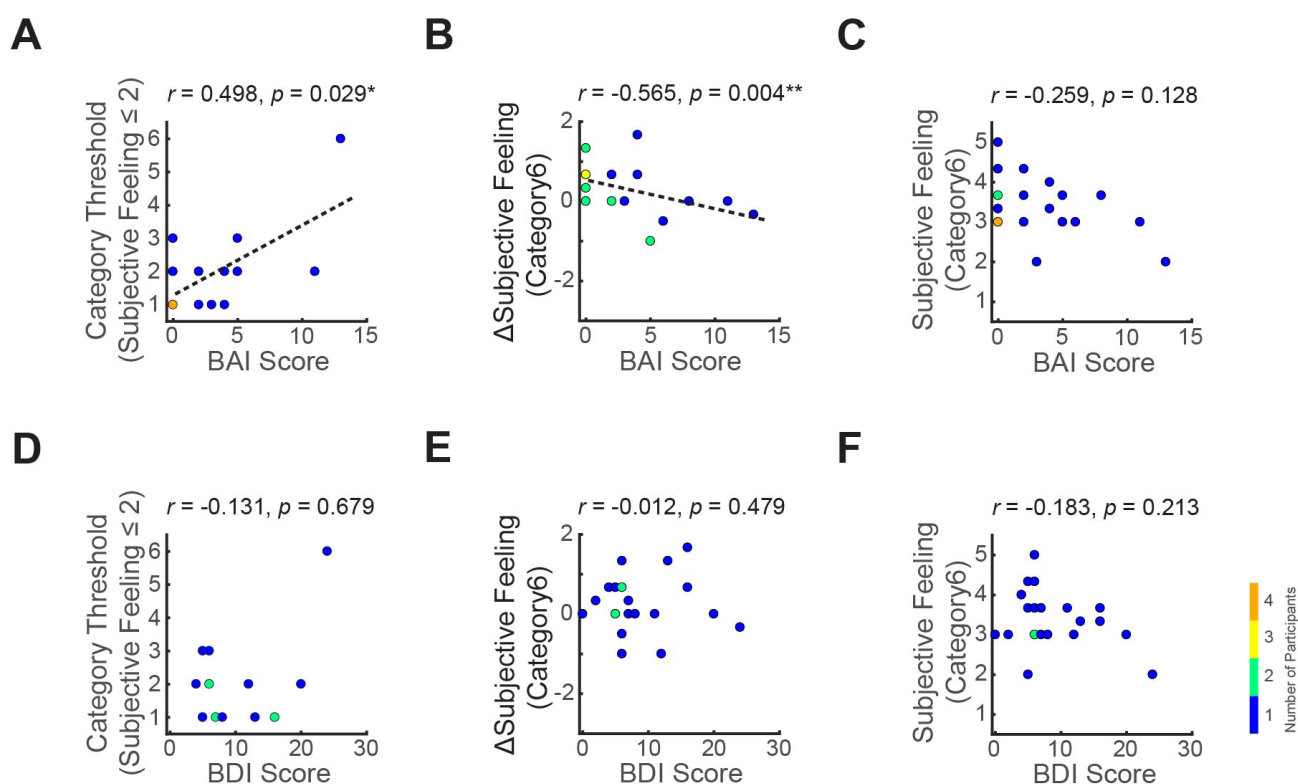


Fig. 3. Relationship between the generalization of conscious feeling induced by fear conditioning and individual anxiety (or depression) level. To determine the degree of fear generalization, we derived the maximum category number of scenes for which the participants rated as score 2 (category threshold) or the change of subjective feeling scores (or average feeling scores during the post-learning test) to scene category 6, which is the last category of GS. (A~C) Correlation between fear generalization and BAI scores. (D~F) Correlation between fear generalization and BDI scores.

20 participants' scores (Fig. 4A and D). Additionally, based on the rationale that a participant with broader fear generalization may show more negative change of scores in the subjective feeling or US-expectancy for the least similar GS with the CS+ after learning, we also measured the change of subjective feeling scores or US-expectancy scores to the scenes of category 6, which is the last category of GS, as an indicator of fear generalization (Fig. 3 and 4). For this analysis, all participant data was used.

Statistical analyses

To compare subjective feeling or US-expectancy scores with basal level (score 3), or to test the difference between post-learning and pre-learning scores, we conducted paired t-tests (two-tailed). Non-parametric Friedman tests (tests of within-subjects effects) were used to determine statistical significance of scene category effect with the assumption that the scores represent an ordinal scale. For correlation analyses, Spearman correlation was used. Statistical analyses were conducted using MATLAB, and SPSS.

RESULTS

Generalization of fear memory

To measure the conscious feeling of fear to each scene image, we asked participants to rate their subjective feeling on a 1~5 scale (1, very unpleasant; 3, moderate; 5, very pleasant) before (pre-learning test) and after (post-learning test) learning (Fig. 1A). Additionally, the participants were also requested to rate US (scream)-expectancy to each scene on a 1~5 scale (1, very likely; 3, not sure; 5, very unlikely) to determine explicit knowledge of the association between scenes and scream. The scores of both ratings were averaged across the three scenes of each category.

The change in subjective feeling induced by fear conditioning was derived by subtracting the feeling scores during the pre-learning test from the scores during the post-learning test (Fig. 1B and 2A). The change of the feeling scores to the CS+ category (category 1) scenes was significantly negative ($t_{(20)} = -3.635, p < 0.005$, two-tailed), indicating that unpleasant feeling to CS+ category scenes significant induced by fear learning (Fig. 2A). The change of feeling scores to CS- category scenes was comparable to zero ($t_{(20)} = 1.117, p = 0.277$, two-tailed) (Fig. 2A). Moreover, we found gradual change of scores from category 1 to 7 (Fig. 2A). The feeling scores to the

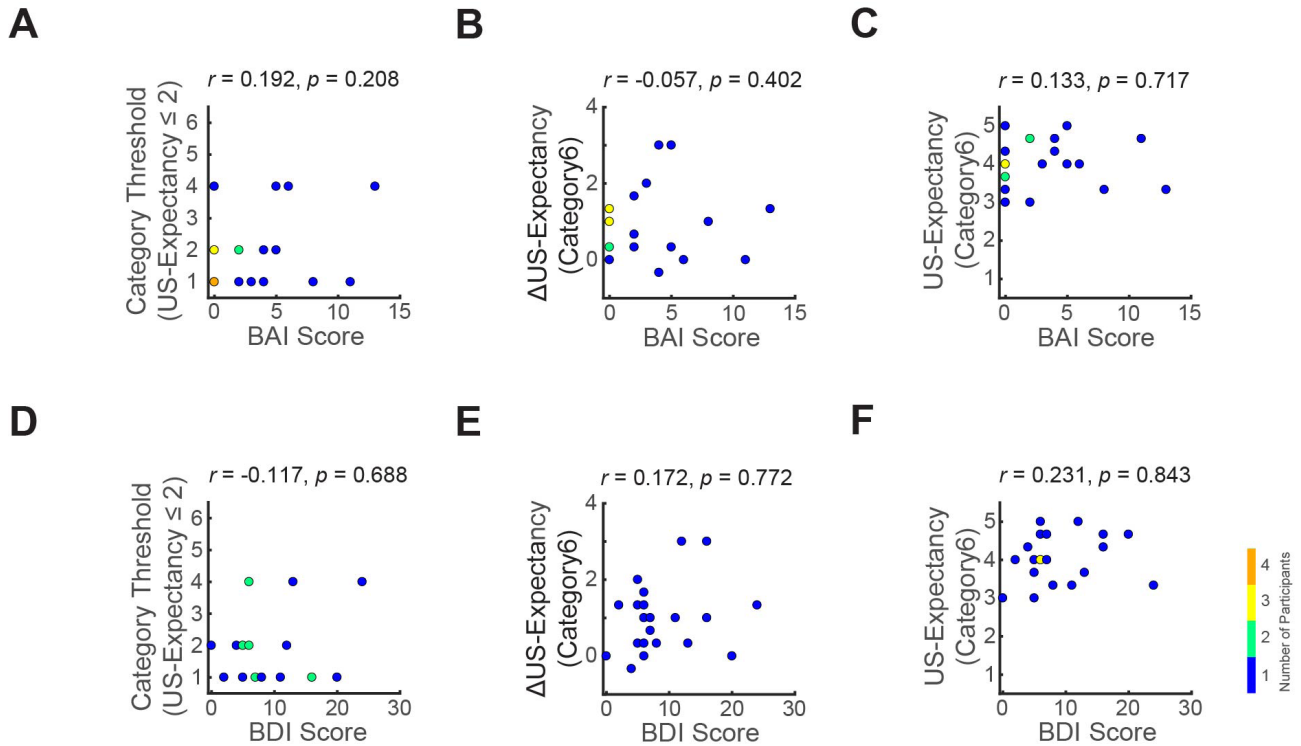


Fig. 4. Relationship between the generalization of US-expectancy and individual anxiety (or depression) level. To determine the degree of fear generalization, we derived the maximum category number of scenes for which the participants rated as score 2 (category threshold) or the change of US-expectancy scores (or average US-expectancy scores during the post-learning test) to category 6 scenes. (A~C) Correlation between fear generalization and BAI scores. (D~F) Correlation between fear generalization and BDI scores.

category 2, 3, and 4 also showed decreasing pattern after learning (category 2: $t_{(20)}=-2.188$, $p<0.05$; category 3: $t_{(20)}=-1.033$, $p=0.314$; category 4: $t_{(20)}=-2.232$, $p<0.05$, two-tailed) whereas the scores to category 5, 6 did not change (category 5: $t_{(20)}=1.363$, $p=0.188$; category 6: $t_{(20)}=1.724$, $p=0.100$, two-tailed) (Fig. 2A). A Friedman test with scene category as within-subject factor revealed the significant effect of scene category on the change of subjective feeling ($\chi^2_{(6, N=21)}=21.841$, $p<0.005$). These results suggest that the participants successfully learned the conditioned fear, and that the unpleasant feeling induced by fear conditioning to CS+ can be generalized to closer category scenes.

In the change of US-expectancy, we also found significantly negative scores to CS+ scenes ($t_{(20)}=-5.452$, $p<0.001$, two-tailed) whereas the scores were significantly positive to CS- scenes ($t_{(20)}=4.963$, $p<0.001$, two-tailed) (Fig. 2B). This means that the participants expected the occurrence of US when they saw CS+ scenes while they anticipated no scream in the presence of CS- scenes. Moreover, the participants did not expect the occurrence of US when the category 5 and 6 scenes were presented after learning (category 5: $t_{(20)}=3.201$, $p<0.005$; category 6: $t_{(20)}=4.958$, $p<0.001$, two-tailed). In addition, a Friedman test revealed statistical significant effect of scene category on the change of US-expectancy ($\chi^2_{(6, N=21)}=48.053$, $p<0.001$). Although the change of feeling to category 2 and 4 scenes was significantly negative (Fig. 2A), there was no change of US-expectancy to the same category scenes (category 2: $t_{(20)}=-0.581$, $p=0.568$; category 3: $t_{(20)}=0.474$, $p=0.640$; category 4: $t_{(20)}=1.037$, $p=0.312$, two-tailed) (Fig. 2B), suggesting that negative feeling can be generalized even when the likelihood of US occurrence is not clear.

In the subjective feeling or US-expectancy scores during the pre-learning test, a Friedman test did not show any significant effect of scene category ($\chi^2_{(6, N=21)}=7.240$, $p=0.299$, for subjective feeling scores; $\chi^2_{(6, N=21)}=9.925$, $p=0.128$, for the US-expectancy scores), although there was slight preference for the scenes of category 3 (two-tailed t-test compared to score 3, $t_{(20)}=2.515$, $p<0.05$) and 4 ($t_{(20)}=3.568$, $p<0.05$) in subjective feeling scores, and for the category 4 in US-expectancy scores ($t_{(20)}=2.156$, $p<0.05$) (Fig. 2C and D).

We also examined the scores during the post-learning test. Consistent with the change of subjective feeling and US-expectancy, the scores during the post-learning also gradually increased from the category 1 to 7 for both feeling and expectancy (Fig. 2E and F). A Friedman test with scene category as within-subject factor revealed the effect of scene category in subjective feeling ($\chi^2_{(6, N=21)}=32.186$, $p<0.001$) and US-expectancy ($\chi^2_{(6, N=21)}=67.776$, $p<0.001$). The average feeling score to the CS+ scene category (category 1) was significantly below 3 (neutral) ($t_{(20)}=-3.191$, $p<0.005$,

two-tailed), indicating significant unpleasant feeling to CS+ scene category (Fig. 2E). For the category 5, 6, and 7, the feeling scores were significantly greater than score 3 (category 5: $t_{(20)}=2.834$, $p<0.05$; category 6: $t_{(20)}=2.343$, $p<0.05$; category 7: $t_{(20)}=3.190$, $p<0.005$, two-tailed) (Fig. 2E). In addition, the average US-expectancy scores were also significantly lower than 3 for the CS+ category ($t_{(20)}=-7.623$, $p<0.001$, two-tailed) and higher than 3 for the category 4, 5, 6, and 7 (category 4: $t_{(20)}=2.256$, $p<0.05$; category 5: $t_{(20)}=6.725$, $p<0.001$; category 6: $t_{(20)}=7.703$, $p<0.001$; category 7: $t_{(20)}=6.200$, $p<0.001$, two-tailed) (Fig. 2F).

Correlation between the generalization of conscious feeling of fear and anxiety level

Anxiety patients show overgeneralization of conditioned fear [27]. To determine whether generalization of conscious feeling induced by fear conditioning depends on anxiety level generally in nonpatient participants, we derived the maximum category number of scenes for which the participants rated as score 2 ('unpleasant'). We defined this maximum category as category threshold (see details in Materials and Methods). To estimate individual anxiety level, we asked participants to answer to BAI questionnaire, and they all showed normal range of BAI scores, which is less than 15 (Fig. 3). We found that the category threshold was positively correlated with BAI scores ($r=0.498$, $p=0.029$, Spearman) (Fig. 3A). Thus, the participant who has the BAI score higher felt unpleasant for the scenes of the broader category.

A participant with broader fear generalization may show more negative change of scores in the subjective feeling for the least similar GS with the CS+ after learning. Thus, we additionally examined whether the change of subjective feeling score to scene category 6, which is the last category of GS, is correlated with BAI scores of the participants. Consistent with the category threshold data, we found negative correlation between the change of subjective feeling to the category 6 and the BAI scores ($r=-0.565$, $p=0.004$, Spearman) (Fig. 3B). This result supports the category threshold data (Fig. 3A), indicating that the participant with the higher BAI score feels worse for the category 6 scenes, which are least similar to the CS+ scenes in GSs, after learning. This negative correlation was pronounced in the change of subjective feeling rather than the feeling scores to the category 6 during post-learning test (Fig. 3C).

Because anxiety disorders and depression frequently co-occur [22, 23], we also examined the correlation between individual depression level with the category threshold, the change of feeling to the category 6, and the subjective feeling to the category 6 scenes during the post-learning test (Fig. 3D~F). However, there was no significant correlation between them (Fig. 3D~F). In addition, we did not find any significant effect in the same correlation analyses

involving US-expectancy for both anxiety and depression levels (Fig. 4). These results suggest that generalization of fear feeling depends on general anxiety level but not depression level. Moreover, this dependency was pronounced in the subjective feeling but weak in US-expectancy.

Correlation between the strength of conscious feeling of fear and depression level

Given the possibility that fear discrimination is related to the strength of fear conditioning [28, 29], we additionally examined the correlations between subjective feeling to CS+ category (category 1) scenes and BAI scores as well as BDI scores (Fig. 5). Whereas either the change of feeling to the category 1 scenes or the feeling to the category 1 scenes during the post-learning test was not significantly correlated with the individual BAI scores (Fig. 5A and 5B), there was significantly negative correlation between the change of feeling and BDI scores ($r = -0.379, p = 0.045$, Spearman) or between the feeling to the category 1 and BDI scores ($r = -0.482, p = 0.013$, Spearman) (Fig. 5C and 5D). Thus, the participant with a higher BDI score felt worse feeling to the CS+ category scenes after learning. The significant correlation was not observed between the BDI scores and US-expectancy for CS+ scenes (Fig. 6). Thus, these results suggest that the strength of fear feeling induced by fear conditioning was mainly related to the individual depression level.

DISCUSSION

Our findings demonstrate that conscious feeling of fear induced by fear conditioning can be generalized to similar contexts, and that this generalization depends on the individual anxiety level in nonpatient participants. Additionally, while the strength of fear conditioning is positively correlated with the individual depression level, the degree of fear generalization in subjective feeling does not depend on the individual depression level.

Despite conscious feeling of fear is directly related to the suffering of the anxiety patients, most studies have focused on the defensive physiological responses but little on the conscious feeling of fear. Our results provide evidence for generalization of conscious fear. Combined with the results of prior studies [7, 15], our findings suggest that not only generalization of defensive physiological responses but also generalization of conscious fear depends on the individual anxiety level. Future work is needed to understand how the process underlying generalization of conscious fear interacts with the process underlying generalization of defensive physiological responses.

Previously, Lissek et al. [14] developed a paradigm of fear generalization using rings of gradually increasing size, and showed generalization of conditioned fear-potentiated startle. Consistent with this, our results showed that conscious feeling of fear can be

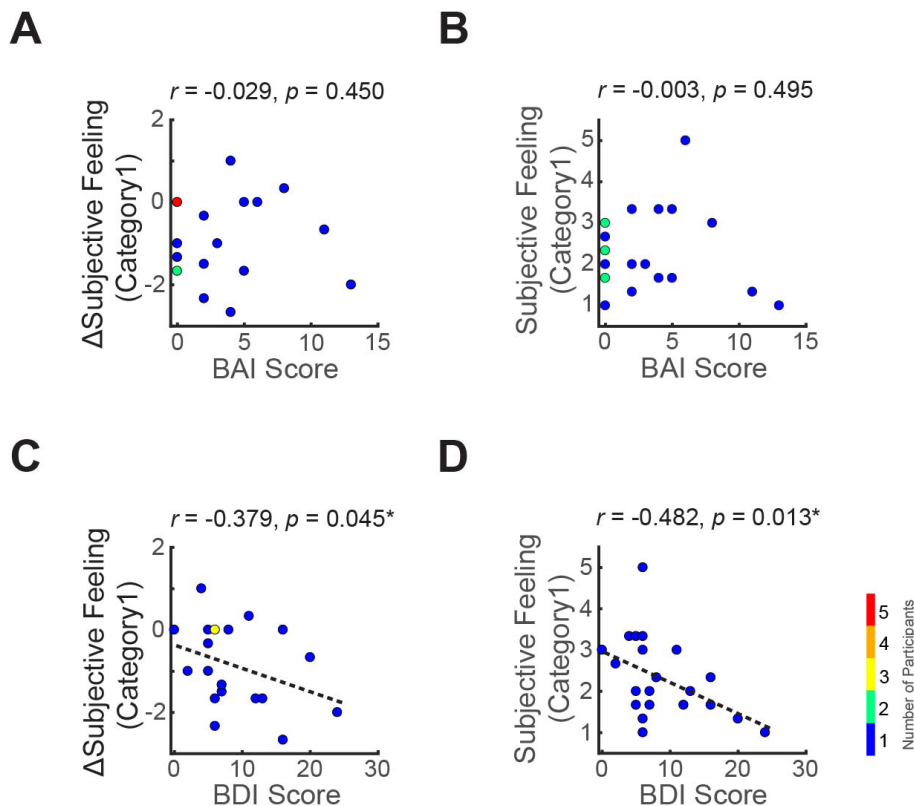


Fig. 5. Relationship between the strength of conscious feeling induced by fear conditioning and anxiety (or depression) level. To determine the strength of fear memory, the change of the feeling scores (or the average feeling scores during the post-learning test) to category 1 scenes (CS+ scenes) were used. (A, B) Correlation between the strength of fear memory and BAI scores. (C, D) Correlation between the strength of fear memory and BDI scores.

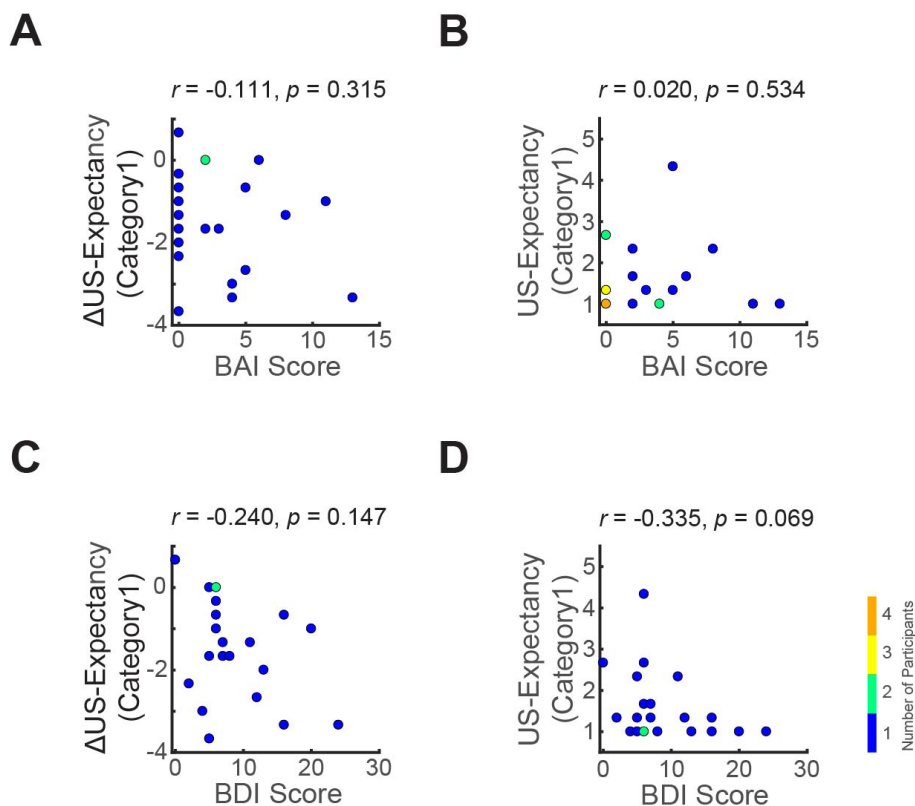


Fig. 6. Relationship between the strength of US-expectancy and anxiety (or depression) level. To determine the strength of fear memory, the change of the US-expectancy scores (or the average US-expectancy scores during the post-learning test) to category 1 scenes (CS+ scenes) were used. (A, B) Correlation between the strength of fear memory and BAI scores. (C, D) Correlation between the strength of fear memory and BDI scores.

generalized to similar contexts with CS+. For this, we used natural scene images as CSs (CS+ and CS-) and GSs for the fear conditioning and fear generalization testing. This scene-based paradigm provides an experimental condition that is close to real-world context. Moreover, this paradigm provides useful tool to investigate whether conscious fear memory including conscious feeling as well as explicit knowledge about the US-CS association can be generalized to semantically similar contexts even when physical properties of images are quite different.

Prior studies have reported that anxiety patients show reduced discrimination between CS+ and CS- in the typical fear conditioning [10, 11]. In addition, the similar impairment of discrimination was observed in anxiety-prone people [30]. Lissek et al. [7] using fear generalization paradigm with gradually changing rings showed overgeneralization of conditioned fear in anxiety patient compared to normal controls. Thus, these results suggest that the patients with anxiety problems show broad range of generalization. Our results extend these prior data, showing that the degree of fear generalization depends on individual anxiety level even in nonpatient participants. Thus, our data suggests that fear overgeneralization observed in anxiety patients is not a distinctive feature of the anxiety patients, but is a result of a general phenomenon dependent on anxiety level. For a future study, it will be interesting to investigate our finding can be applied to both trait anxiety (anxiety

level as a personal temperament) and state anxiety (anxiety about an event) [31, 32].

While generalization of subjective feeling was correlated with the anxiety level, the correlation between the US-expectancy and the anxiety level was weak (Fig. 4A~C). This may be due to different degree of fear generalization between subjective feeling and US-expectancy. The participants were unpleasant to the scenes that are in close category to CS+ even when they are not sure the occurrence of US (Fig. 2A and B). The low gradient across the scene categories in the change of subjective feeling compared to the change of US-expectancy may result in more sensitive reflection of the individual anxiety level. Future studies are needed to investigate why fear generalization is pronounced in the subjective feeling.

Given that depression frequently co-occurs with anxiety disorders [22, 23, 30], the individual depression level might be also correlated with the degree of fear generalization. However, we did not find any significant relationship between the depression level and generalization of subjective feeling or US-expectancy (Fig. 3D~F and 4D~F). Indeed, consistent with our results, other studies have reported that depression patients or depression-prone persons showed enhanced CS+ conditioning in skin conductance response [28, 30]. It will be interesting to further investigate the neural substrates underlying differential phenomenon between anxiety and depression levels in relation to fear generalization.

In conclusion, the present results show that subjective feeling of fear is generalized to similar context, and this generalization depends on individual anxiety level but not depression. These results provide evidence supporting that generalization of the conscious feeling induced by fear conditioning generally depends on individual anxiety level.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare (HI15C3175), the Basic Science Research Program (NRF-2016R1C1B2010726), and the Brain Research Program (NRF-2017M3C7A1031333) through the National Research Foundation (NRF) of Korea, and the Undergraduate Research Program of KAIST. Thanks to H. E. Kim and members of the Memory and Cognition Laboratory, KAIST for helpful discussion.

REFERENCES

1. Onat S, Büchel C (2015) The neuronal basis of fear generalization in humans. *Nat Neurosci* 18:1811-1818.
2. Dunsmoor JE, Paz R (2015) Fear generalization and anxiety: behavioral and neural mechanisms. *Biol Psychiatry* 78:336-343.
3. Rosen JB, Schulkin J (1998) From normal fear to pathological anxiety. *Psychol Rev* 105:325-350.
4. Foa EB, Kozak MJ (1986) Emotional processing of fear: exposure to corrective information. *Psychol Bull* 99:20-35.
5. Dymond S, Dunsmoor JE, Vervliet B, Roche B, Hermans D (2015) Fear generalization in humans: systematic review and implications for anxiety disorder research. *Behav Ther* 46:561-582.
6. Anastasides N, Beck KD, Pang KC, Servatius RJ, Gilbertson MW, Orr SP, Myers CE (2015) Increased generalization of learned associations is related to re-experiencing symptoms in veterans with symptoms of post-traumatic stress. *Stress* 18:484-489.
7. Lissek S, Rabin S, Heller RE, Lukenbaugh D, Geraci M, Pine DS, Grillon C (2010) Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *Am J Psychiatry* 167:47-55.
8. Mineka S, Zinbarg R (2006) A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am Psychol* 61:10-26.
9. Watson JB, Rayner R (1920) Conditioned emotional reactions. *J Exp Psychol* 3:1-14.
10. Lissek S, Rabin SJ, McDowell DJ, Dvir S, Bradford DE, Geraci M, Pine DS, Grillon C (2009) Impaired discriminative fear-conditioning resulting from elevated fear responding to learned safety cues among individuals with panic disorder. *Behav Res Ther* 47:111-118.
11. Sachs G, Anderer P, Doby D, Saletu B, Dantendorfer K (2003) Impaired conditional discrimination learning in social phobia. *Neuropsychobiology* 47:66-72.
12. Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS (2005) Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behav Res Ther* 43:1391-1424.
13. Jovanovic T, Kazama A, Bachevalier J, Davis M (2012) Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology* 62:695-704.
14. Lissek S, Biggs AL, Rabin SJ, Cornwell BR, Alvarez RP, Pine DS, Grillon C (2008) Generalization of conditioned fear-potentiated startle in humans: experimental validation and clinical relevance. *Behav Res Ther* 46:678-687.
15. Lissek S, Kaczkurkin AN, Rabin S, Geraci M, Pine DS, Grillon C (2014) Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. *Biol Psychiatry* 75:909-915.
16. Gluck MA, Mercado E, Myers CE (2013) Emotional influences on learning and memory. In: *Learning and memory: from brain to behavior*. 2nd ed (Gluck MA, Mercado E, Myers CE, eds), pp 385-426. Worth Publishers, New York, NY.
17. Lang PJ, McTeague LM (2009) The anxiety disorder spectrum: fear imagery, physiological reactivity, and differential diagnosis. *Anxiety Stress Coping* 22:5-25.
18. Labar KS, Ledoux J (2003) Emotion and the brain: an overview. In: *Behavioral neurology and neuropsychology*. 2nd ed (Feinberg TE, Farah MJ, eds), pp 711-724. McGraw-Hill, New York, NY.
19. Cannon WB (1927) The James-Lange theory of emotions: a critical examination and an alternative theory. *Am J Psychol* 39:106-124.
20. Tinoco-González D, Fullana MA, Torrents-Rodas D, Bonillo A, Vervliet B, Blasco MJ, Farré M, Torrubia R (2015) Conditioned fear acquisition and generalization in generalized anxiety disorder. *Behav Ther* 46:627-639.
21. Greenberg T, Carlson JM, Cha J, Hajcak G, Mujica-Parodi LR (2013) Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. *Depress Anxiety* 30:242-250.
22. Gorman JM (1996-1997) Comorbid depression and anxiety

- spectrum disorders. *Depress Anxiety* 4:160-168.
23. Pollack MH (2005) Comorbid anxiety and depression. *J Clin Psychiatry* 66 Suppl 8:22-29.
 24. Xiao J, Hays J, Ehinger KA, Oliva A, Torralba A (2010) SUN database: large-scale scene recognition from abbey to zoo. *Proc IEEE Comput Soc Conf Comput Vis Pattern Recognit* 3485-3492.
 25. Beck AT, Steer RA, Brown GK (1996) Manual for the Beck depression inventory-II. Psychological Corporation, San Antonio, TX.
 26. Beck AT, Epstein N, Brown G, Steer RA (1988) An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol* 56:893-897.
 27. Lissek S, Grillon C (2010) Overgeneralization of conditioned fear in the anxiety disorders. *J Psychol* 218:146-148.
 28. Nissen C, Holz J, Blechert J, Feige B, Riemann D, Voderholzer U, Normann C (2010) Learning as a model for neural plasticity in major depression. *Biol Psychiatry* 68:544-552.
 29. Orr SP, Metzger LJ, Lasko NB, Macklin ML, Peri T, Pitman RK (2000) De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *J Abnorm Psychol* 109:290-298.
 30. Dibbets P, van den Broek A, Evers EA (2015) Fear conditioning and extinction in anxiety- and depression-prone persons. *Memory* 23:350-364.
 31. Kvaal K, Ulstein I, Nordhus IH, Engedal K (2005) The Spielberger state-trait anxiety inventory (STAI): the state scale in detecting mental disorders in geriatric patients. *Int J Geriatr Psychiatry* 20:629-634.
 32. Spielberger CD (1989) State-trait anxiety inventory: a comprehensive bibliography. 2nd ed. Consulting Psychologists Press, Palo Alto, CA.