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STATE ANXIETY COULD INCREASE DISCRIMINATION IN HUMAN FEAR CONDITIONING

EL ESTADO ANSIOSO PODRÍA MEJORAR LA DISCRIMINACIÓN EN CONDICIONAMIENTO AL MIEDO EN HUMANOS

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Abstract: Evidence has shown that individuals with anxiety disorders show more intense fear responses to both stimuli signaling threat and stimuli representing safety. The latter often causes difficulty to learn fear inhibition. This study aimed to assess the role of state anxiety in fear acquisition and extinction. During fear conditioning, geometric figures served as conditioned stimuli and a mild electric shock as unconditioned stimulus. Unconditioned stimulus expectancy ratings were used to assess fear. Results showed that high state anxiety is associated with higher responses to stimuli predicting the aversive stimulus and lower responses to stimuli not predicting it, suggesting that individuals in a high anxiety state have a larger fear activation to danger cues and lower activation to safety cues.

Keywords: individual differences, trait vulnerability, differential conditioning, exposure therapy, quasi-experiment, STAI.

Resumen: La evidencia ha demostrado que los individuos con trastornos de ansiedad muestran respuestas de miedo más intensas tanto a estímulos que señalan una amenaza como a aquellos que representan seguridad. Lo anterior se traduce generalmente en una dificultad para aprender a inhibir el miedo. El propósito de este estudio fue investigar el papel del estado ansioso en la adquisición y extinción del miedo. Durante el condicionamiento del miedo, figuras geométricas funcionaron como estímulos condicionados y un electrochoque suave como estímulo incondicionado. Se midió el miedo mediante la expectativa del estímulo aversivo. Los resultados indican que los individuos con estado ansioso alto presentan una mayor respuesta a estímulos que predicen el estímulo aversivo y menores respuestas a estímulos que no lo predicen. Lo encontrado sugiere que los individuos en estado de ansiedad tienen una mayor activación del miedo en presencia de claves que señalan peligro y menor activación a claves de seguridad.

Palabras clave: diferencias individuales, vulnerabilidad del rasgo, condicionamiento diferencial, terapia de exposición, cuasiexperimento, STAI.

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Anxiety, a generalized alert state to an unspecific threat (Spielberger, 2010), is an adaptive response to threats or potentially harmful stimuli in the natural environment. However, under certain conditions, some individuals can experience a long-lasting response that can become maladaptive. Anxiety disorders are characterized by overgeneralization of fear to stimuli that do not represent a real threat. Difficulties to inhibit fear in the presence of safety cues are also described (Andreatta & Pauli, 2017; Craske, 2015; Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015).

The effects of anxiety on the generalization of fear can be examined during learning new fear responses through fear conditioning (Maples-Keller, Yasinski, Manjin, & Rothbaum, 2017). In fear conditioning, individuals are exposed to pairings of initially neutral stimulus (e.g., a geometric figure; conditioned stimulus or CS) with a consequence (e.g., a bite; unconditioned stimulus or US). Individuals learn to respond to the CS through these pairings (e.g., increase in skin conductance response and US expectancy in front of the geometric figure; conditioned response). This paradigm is a useful tool to analyze the effect of anxiety because is the basis of many anxiety disorders, including specific phobia, social phobia, panic disorder, among others (Craske, Hermans, & Vervliet, 2018; Laborda, Miguez, Polack, & Miller, 2012; Quezada et al., 2018).

The evidence suggests that anxiety disorders might enhance the acquisition of new conditioned response of fear in front of danger cues, and could have some difficulty in diminishing this conditioned response during extinction in a single-cue conditioning design (Lissek et al., 2005). Furthermore, anxiety disorders appear to be associated with failures to inhibit fear responses to cues that do not signal a threat, for example, by increasing the conditioned fear response to conditioned safety cues (CS–) during acquisition of fear conditioning. Likewise, anxious individuals show higher responses to cues that no longer represent danger, as when previously excitatory CS (CS+) are presented without the US, in a process known as extinction. In addition, patients tend to show greater differentiation in the responses to CS+ and CS– during extinction (Duits et al., 2015). Given that anxious disorders have effects on learning and the extinction of fear, it is interesting to study if the risk factors for the development of anxiety disorders could replicate the same effects, which would lead to a greater understanding of pathological anxiety. In the field of human fear conditioning, current research shows that trait and state anxiety constitute an important vulnerability for anxiety disorders (Chambers, Power, & Durham, 2004; Grillon, Dierker, & Merikangas, 1998). Trait anxiety is a relatively enduring disposition to feel stress, worry, and discomfort. State anxiety corresponds to fear, nervousness, discomfort, and arousal of the autonomic nervous system induced temporarily by situations perceived as dangerous (Spielberger & Sydeman, 1994). Considering the defining features of state and trait anxiety, it is likely that different processes might influence the generalization of fear. Notably, much research has focused on the effect of trait anxiety (Boddez et al., 2012; Torrents-Rodas et al., 2013) as a more permanent feature, on the generalization of fear conditioning and inhibition, leaving state anxiety somewhat forgotten and understudied.

Some studies have shown that trait anxiety could affect the learning of inhibition. Gazendam, Kamphuis, and Kindt (2013) compared high trait anxious individuals to healthy controls in a fear conditioning task, concluding that trait anxious participants showed an impairment in safety learning, measured as a higher response to stimuli that are not related to the aversive consequence. Boddez et al. (2012) on the other hand, examined the relationship between trait anxiety and blocking of aversive conditioning in humans. They found that trait anxiety was associated with the expectation of occurrence of the shock to a blocked stimulus. This suggests that trait anxiety plays an important role in the acquisition and extinction and/or inhibition of fear.

Other studies, however, have reported no difference between acquisition and generalization of fear among patients diagnosed with generalized anxiety disorder compared to healthy controls (Tinoco-González et al., 2015), and in the acquisition and generalization of fear among adults with either low, medium or high levels of trait anxiety (Torrents-Rodas et al., 2013). Furthermore, Kindt and Soeter (2014) compared students with high and low trait anxiety on a conditional discrimination procedure. They observed impaired inhibitory learning only on the cognitive level, but not in the startle reflex.

More central to the focus of the present study, are the findings reported by Liao and Craske (2013). They induced either a state of high or low anxiety to participants and compared their expectations of a threat to a CS– presented simultaneously with a CS+. They found that state anxiety had an interfering influence in the inhibition of fear. In the Liao and Craske's study, trait anxiety is recognized as a preexisting condition while state anxiety is induced. Trait anxiety present among participants beforehand may have a different effect to induced anxiety. Moreover, given that state anxiety involves arousal, which is a component

of motivation that directly affects learning (Bailey et al., 2015), a higher state anxiety may enhance discriminatory fear learning instead of impairing it.

Also of interest to the present research, Dibbets and Evers (2017) related the level of state anxiety (as a continuous variable) with US expectancy in a fear conditioned experiment. They found that participants with higher state anxiety levels were significantly worse discriminating between a reinforced and a non-reinforced CS in a differential conditioning protocol. Importantly, the state anxiety measure in this experiment was taken after a stress-inducing task, similar in that matter to Liao and Craske's (2013) study.

Considering that there is considerably less evidence regarding the effect of state anxiety on the acquisition and extinction of fear conditioning as compared to trait anxiety, and that the two different types of anxiety could be associated with different effects on fear learning, the aim of the present study was to examine the relationship between state anxiety in the acquisition and extinction of fear in an experimental setting. Healthy participants responded the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) before receiving pairings of a CS with a mild electric shock and non-reinforced presentations of another CS. If state anxiety is underlined by a different mechanism to those of trait anxiety, a higher state anxiety that could enhance differential conditioning was expected instead of just increasing fear responses.

METHOD

Participants

A total of ninety-six university students (51 female; mean age = 22.18, *SD* = 4.08, range 18-34), participated in this experiment. Participants received a coupon for photocopies or a ticket for a movie as a compensation for their collaboration and time spent on the study. To be included in the experiment they must be right handed, be within the cohort range score in the psychological dimensions evaluated by the Symptom Checklist-90-Revised (SCL-90-R; Derogatis, 1992) and/or did not have a medical history that justified exclusion (evaluated by a medical checklist). The exclusion criteria considered the participant who was currently under psychological treatment for one of the main diagnostic categories of the *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (American Psychiatric Association, 2014), with a history of psychiatric hospitalization, coronary heart disease, and drug or alcohol consumption 24 hours prior to the realization of the experiment. All subjects signed an informed consent and all procedures were approved by the Ethics Committee of Research in Social Sciences of the Universidad de Chile.

Instruments

The SCL-90-R (Derogatis, 1975) is a ninety items self-report questionnaire which assesses nine symptomatic dimensions related to psychological distress experimented by a person: Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation and Psychoticism. The Chilean normative data and psychometric properties obtained by Gempp Fuentealba and Avendaño Bravo (2008) were used in the present study. This adapted version presents an acceptable congruence between the obtained factorial matrix and the original, with a Euclidean Similarity Index for the global scale of .87. The nine scales have appropriate reliability indices (α values between .64 and .82). The questionnaire was filled out by the participants prior to being included in the experiment.

The STAI (Spielberger et al., 1970) is a self-report questionnaire that contains two subscales of twenty items each, which allow to evaluate the levels of anxiety in two components, State (STAI-S) and Trait (STAI-T). The Chilean validated version (Vera-Villarroel, Celis-Atenas, Córdova-Rubio, Buela-Casal, & Spielberger, 2007) was used. This reported a high internal consistency for STAI-S (α = .92) and for STAI-T (α = .87). Participants responded the questionnaire before the beginning of the experiment. Afterwards, they were assigned to one of three categories according to their state anxiety levels based on the score obtained in each scale (low anxiety: percentiles 1 to 25; medium anxiety: percentiles 35 to 65; high anxiety: percentiles 75 to 100).

Stimuli and Apparatus

Three geometric figures, a square (5 cm per side), an equilateral triangle (5 cm per side) and a circle (6 cm diameter) were used as CS; X, Y and Z (counterbalanced). These were presented on a computer screen to participants. The presence of Y+ and Z– during the acquisition allows controlling the effects of orientation and habituation produced by the repeated presentation of a single stimulus (Vansteenwegen

et al., 2006), and at the same time, models a more complex conditioning situation, which increases the variance in the response due to individual differences in anxious vulnerability (Lissek, Pine, & Grillon, 2006).

A and B were different contexts in which the acquisition and extinction phases occurred. This was composed of different background screen colors on which the figures and cues were shown. Context A was a blue background (RGB: 102, 153, 204), and remained constant for all participants. Context B was either a red (RGB: 195, 63, 30) or a green (RGB: 34, 177, 76) screen. Different contexts were used during both phases to eliminate any potential fear to the acquisition context, and to equate the design to a more naturalistic setting where fear and extinction occur in different context and two different colors were used to make it more similar to designs of recovery after extinction (Díaz, Quezada, Navarro, Laborda, & Betancourt, 2017). The US was a mild but uncomfortable electric shock on the left forearm, with a pulse train duration of 200 ms (2 ms pulse at a frequency of 250 Hz) and intensity varying from 8 to 24 mA (Díaz et al., 2017; Quezada et al., 2018). Participants were asked to select an intensity that was unpleasant and demanded some effort to tolerate (Spoormaker et al., 2010; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008; Vervliet & Geens, 2014). The delivery of the electric shock was controlled by a Digitimer DS7A Constant Current Stimulator (Hertfordshire, UK) via a pair of steel disk electrodes of 8 mm diameter with 30 mm spacing, located in the forearm.

The conditioned response was defined as the expectancy of an incoming US, measured in real time during each stimulus presentation using a visual analog scale (VAS) of 1-100 range, 108 mm long, located below the stimulus at the bottom of the screen. Participants could click and drag the scale with the mouse to set the indicator anywhere in a horizontal line, for which on the left end of the screen, indicated that the shock was *certainly not expected* (1), and on the right end of the screen that the shock was *certainly expected* (100). The range of values between the two corners signaled intermediate possibilities of US occurrence. The experiment was conducted on a Hewlett-Packard desktop computer interfaced with an Arduino Uno board for shock delivery and programmed using the E-prime software (Psychology Software Tools, http://www.pstnet.com/). The experiment was carried in a sound-isolated room.

Procedure

A summary of the procedure can be found on Figure 1.

State-Trait Anxiety Inventory. Before initiating the task, participants signed the informed consent and answered the STAI, and then were assigned to one of three groups for the analysis (low, n = 24, medium, n = 34, and high, n = 28, state anxiety; this assignment was not informed to the participants). Each participant was then led to the experimental room and seated in a comfortable armchair, while the experimenter connected the electrodes to provide the electric shock. Then, a discharge of 8 mA was administered so the participant could gradually modulate the intensity of the shock (with a maximum of 24 mA) until the subject considered that it was "unpleasant and demand some effort to tolerate". After this step, the experimenter instructed the participant on the use of the VAS, and invited him/her to rate the chances of the US coming in three trials with each of the CS that were used in the next phase; no electrical shocks were given in those trials.

Acquisition. A fear conditioning paradigm adapted from Dibbets, Havermans, and Arntz (2008) was used. During the acquisition phase, each of three stimuli (a black square, a triangle, and a circle) were presented individually 4 times for 8 s in random order with the restriction that the shock should not occur more than two consecutive times. Each presentation of X and Y were followed immediately by the application of the US in the forearm. A third stimulus (Z–) was also presented, after which no shock was applied. The inter-trial interval (ITI) varied randomly between 4 and 24 s (average = 14 s).

Extinction. Immediately after the acquisition phase was completed, the extinction began. During this phase, X and Z were presented without the US (X– and Z–) in a different context to the one used during acquisition. Each stimulus was presented 10 times, with a duration of 8 s for each presentation. The average ITI was 5 s (ranging from 4 to 6 s). The ITI was modified for this phase in order to prevent a decrease in attention in the absence of shocks during extinction.

Statistical Analyses

The average response to the VAS in every trial and specifically on the last trial of acquisition for X+, Y+, and Z–, and the last extinction trial of X– and Z– were compared between the three groups as a function of the levels of state anxiety. A repeated measures ANOVA was carried out, using

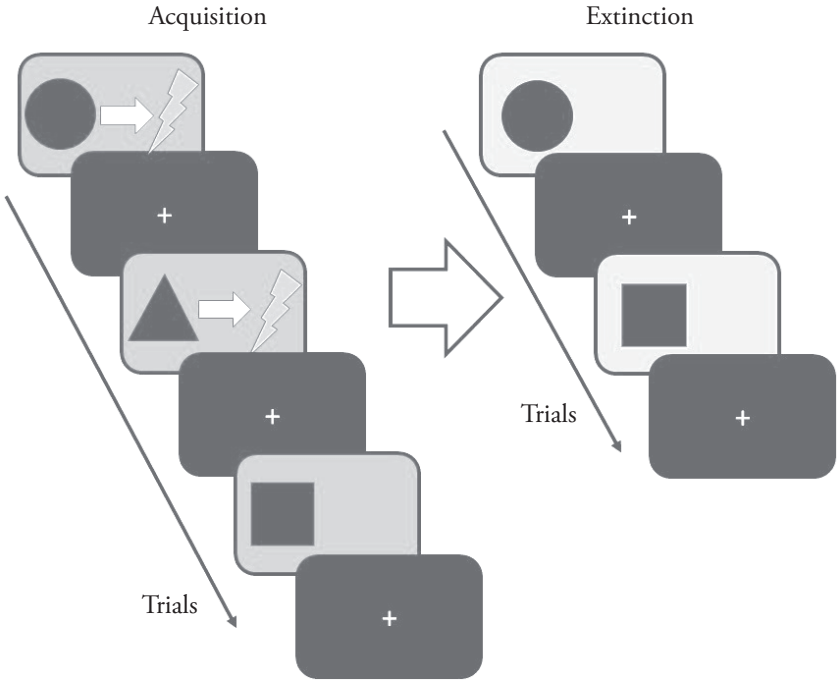

Figure 1. Experimental design. Graphic depiction of the design of the present experiment for a particular subject. Circle, square and triangle (counterbalanced) were used as conditioned stimuli. The electric symbol stands for the 200 ms electrical stimulation in the forearm used as the unconditioned stimulus. The white cross symbolizes the inter-trial interval, which had an average of 20 s during acquisition and 5 s during extinction. The cues were presented on a blue background during acquisition, and a green or red background during extinction.

the different stimuli (X, Y, and Z) as within-subject factors and state anxiety as a between-subject factor. Main effects and Stimulus × Anxiety interaction were evaluated. Non-planned comparisons were Bonferroni corrected. Effect sizes and their confidence intervals are reported as partial eta squared (η^2_p).

RESULTS

The VAS scores for US expectancy in presence of the excitatory cues X and Y were not different during acquisition ($p > .05$), so their data was pooled as one. Mixed ANOVA for all groups on the four acquisition trials and cue (X-Y and Z) showed a main effect of trial, F (1, 83) = 8.31, MSE = 3,394.60, $p = .00$, $\eta^2_p = .048$, and a significant Trial × Cue interaction, F (3, 83) = 96.40, MSE = 39,398.70, $p = .00$, $\eta^2_p = .37$, but no interaction with group of state anxiety ($p > .05$), showing no effect of state anxiety across acquisition trials. VAS scores for X-Y during the last acquisition trial were compared to responses to the non-reinforced stimulus Z to assess differences at the end of acquisition. These scores are presented in Figure 2. The ANOVA showed a main effect of stimulus, F (1, 83) = 275.86, MSE = 221,605.60, $p = .00$, $\eta^2_p = .78$, 95% CIs [80.37, 90.10]-[7.84, 16.39], with a higher average score for the excitatory stimuli (M = 85.25, SD = 23.96) than for the non-reinforced stimulus (M = 12.12, SD = 20.99) during the last trial of acquisition. There was also a significant Stimuli × State Anxiety interaction, F (2, 83) = 3.86, MSE = 3,109.60, $p = .00$, $\eta^2_p = .09$. Post-hoc comparisons showed that participants in the medium and high anxiety groups showed a bigger difference between the excitatory cues and Z– than the participants in the low anxiety group, 95% CI for X-Y-low anxiety: [61.62, 88.05] vs. X-Y-medium anxiety: [86.25, 96.04], X-Y-high anxiety: [83.48, 96.04] vs. Z-low anxiety: [7.13, 29.01], Z-medium anxiety: [2.70, 19.85] vs. Z-high anxiety: [2.69, 9.81]. All other comparisons were not significant, all ps > .05.

The VAS scores during the last trial of the extinction phase for stimuli X and Z were also analyzed. These scores are depicted in Figure 3. There was neither main effect of

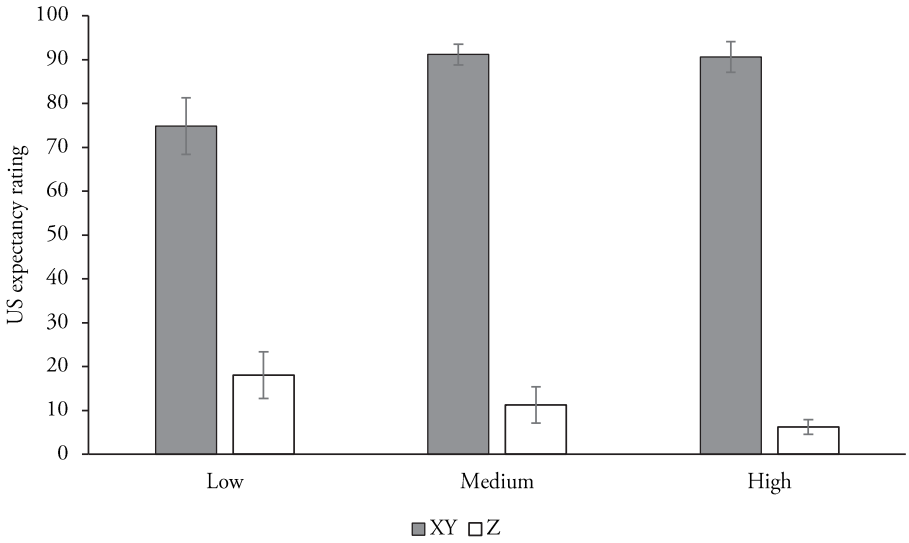

Figure 2. Last acquisition trial differences due to state anxiety. Visual analog scale scores during the last trial of the acquisition phase for reinforced (X and Y) and a non-reinforced (Z) stimuli in a differential conditioning procedure. Scores are presented for subjects with low, medium, or high levels of state anxiety. Error bars represent standard error of the mean.

stimulus or state anxiety nor interaction between these factors, all *p*s > .05. This indicates that the groups were not different at the end of the extinction procedure and that responses to both stimuli did not differ.

DISCUSSION

The present experiment examined how state anxiety levels modulate differential learning between fear-signaling cues and safety cues. Participants were assigned to three different conditions according to their state anxiety levels as indicated by the STAI-S scores. All participants then went through acquisition and extinction of fear conditioning. The results showed that subjects with medium and high anxiety levels presented a greater difference in expectancy between the cues that predicted the shock and the non-reinforced cue than the participants with low anxiety levels. Results showed also no difference between groups in the extinction test.

In previous studies (e.g., Boddez et al., 2012; Duits et al., 2015; Liao & Craske, 2013) individuals with high anxiety levels also showed higher responses to cues that predicted a threat, i.e., presented higher response levels to cues independently of their associative status. The present experiment is consistent with these results. The increase in response to excitatory stimuli in subjects with higher anxiety levels implies that individuals with medium and high state anxiety might have a larger activation of fear in presence of danger-signaling cues (CS+), which would strengthen responses to potential threats. This is coherent with Eysenck's (1979) proposal regarding the development of pathological anxiety; anxiety that would cause excessive fear acquisition among susceptible subjects. Thus, it is predictable that anxious individuals would respond more intensely to signs of a danger than non-anxious subjects.

Regarding cues not paired with the US, however, the results of the present experiment are inconsistent with previous research. Subjects with high levels of anxiety usually show higher responding to safety cues compared to individuals with low anxiety (e.g., Boddez et al., 2012; Duits et al., 2015; Liao & Craske, 2013). In the present experiment, participants with a high level of anxiety showed lower levels of US expectancy to safety cues than subjects with a low level of anxiety. It is likely that since most previous experiments examine the effect of a trait or general anxiety, while in this study we examined the effect of state anxiety, this suggests that state and trait anxiety may involve different underlying processes, which could thereby have different effects on fear learning. An increment in state anxiety involves a general increase in arousal, which may have

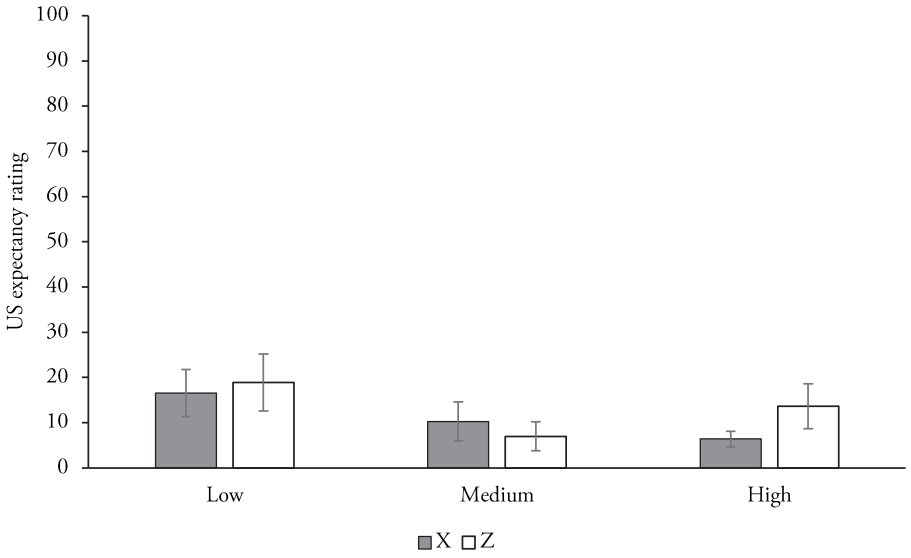

Figure 3. Visual analog scale scores during the last trial of the extinction phase for stimuli X and Z. There are no differences due to state anxiety in last extinction trial. Scores are presented for subjects with low, medium, or high levels of state anxiety. Error bars represent standard error of the mean.

an enhancing effect on learning. This potential difference between state and trait anxiety should be addressed in future experiments.

It is worth noticing that the sample used in this experiment has some features that may help explain the differences with previous research. The average scores in the STAI-S of the present sample are in the lower range within the normative data obtained by Vera-Villarroel et al. (2007) from a sample of Chilean teenagers and adults. This suggests that participants did not reach critical levels of anxiety. Moreover, considering that state anxiety is a transitory condition the levels of anxiety could have diminished during training to an even lower level (Liao & Craske, 2013). This decrement during training would not have occurred in previous experiments in trait anxiety considering that trait is a more long-lasting feature. It is possible that this decrement in anxiety levels across the experiment may account for the absence of any detectable effect of anxiety during the extinction phase.

Considering the generally low state anxiety level of participants in this experiment, future research should try to replicate findings with a representative sample of the general population or who report a wider range of anxiety scores. Future studies should also focus on the potential differences between trait and state anxiety that this experiment suggests, and the possibility that the involvement of arousal in state anxiety might be associated with an enhancing effect of anxiety on learning.

The results of this experiment show that it is relevant to recognize the susceptibility to the development of anxiety disorders in the study of conditioned fear and pathological anxiety at an experimental level, exposing the importance of considering personal differences in the acquisition of fear (Lonsdorf & Merz, 2017). Similarly, prospective longitudinal studies (e.g., Lissek et al., 2005) would be useful to understand whether individual differences in fear conditioning are really a product of the anxious vulnerability of the participants or whether they represent a consequence of prior learning with threatening stimuli.

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