Differences of Emotional Responses to the Stimuli Between Individual With and Without High Depressive Scores

Övgü Peker

Erasmus School of Social and Behavioral Sciences

Erasmus University Rotterdam

Dr. Marta Andreatta

July 30, 2020

Abstract

Stress has an important role in memory process. As stress is affected by depression and also affects depression, in this study I investigated, how the interaction between stress and depression can affect both valence and fear ratings for geometrical shapes (conditioned stimulus, CS+ and other conditioned stimulus, CS-) as a verbal or explicit level and startle potentiation for CS+ and CS- as a physiological or implicit level. One day after either undergoing the Socially Evaluated Cold Pressure Test (SECPT) in stress group or control group which was not exposed to SECPT, the geometrical shape CS+ was presented with painful electric shock in the acquisition phase and there was successful fear acquisition on ratings but not in startle response. In addition, depression and being exposed to stress did not play a significant role in both verbal and physiological responses. On extinction day, the control group had successful extinction learning on fear ratings and there was impaired extinction learning on startle response due to marginal effect as well as valence ratings. Moreover, while it was found that depressed individuals had more fearful ratings to CS+ as compared to nondepressed people at the end of extinction, depression had no significant effect on valence ratings and startle response. Besides, it was clearly found that spontaneous recovery as a verification of the return of fear was observed for valence ratings and startle response independently from depression and group, whereas fear ratings had also same results but not significant. Moreover, there was impaired extinction learning on fear ratings of depressed people in the stress group. On the last day (test day), participants still remembered that CS+ was more negative than CS- at the beginning and at the end of the test. Likewise, for fear ratings, they remember the difference between CS+ and CS- independently from time and group. On the contrary, it was found that there was equal startle response to CS+ and CS-. Being exposed to stress and depression did not have significant effect on ratings and startle response. There was only marginal but not significant effect showing that depressed people in the stress group rated CS+ more fearful as compared to CS-.

Introduction

Major Depressive Disorder (MDD), or clinical/unipolar depression (Rajkowska, 2003), is considered an important medical condition, which damages the well-being of individuals, their work and social life (Caverzasi et al.,2012; Griffiths et al., 2009). It is characterized by a change in mood, self-punitive wishes like dying or escaping, a negative self-concept related to self-blame, vegetative changes (reduction or increase in appetite, loss of libido or insomnia) and alterations in activity level such as moving slowly or agitation (Alford & Beck, 2014).

In addition to this definition, according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013), individuals should have at least five of the following symptoms for the duration of at least two weeks to be diagnosed with Major Depressive Disorder. These symptoms are depressed mood most of the day, significant weight loss or gain weight, diminished ability to think, concentrate or indecisiveness, fatigue, psychomotor agitation or retardation, diminished pleasure or interest, insomnia or hypersomnia, feelings of worthlessness or inappropriate guilt and recurrent thoughts of death. In addition, DSM-5 indicates that the symptoms of MDD should cause impairments or serious distress in occupational, social or other areas of functioning in the lives of people with MDD.

Caverzasi et al., (2012) reported that MDD is considered as one of the most frequently diagnosed psychiatric disorder. According to DSM-5, in the United States, the twelve-month prevalence of clinical depression is approximately 7% and it changes according to age and gender. For instance, in the beginning, the early adolescence, the prevalence in females is 1.5 to 3 fold higher rates than males or 18 to 29 years old people experience clinical depression 3 times higher than 60 years old or older individuals.

In addition to this high prevalence of clinical depression, there are too many healthy people who express themselves as depressed when they have any lower mood than their baseline or feel transient loneliness or sadness even if they are not in depression (Alford & Beck, 2014). However, to be diagnosed clinicians should use the standard method of diagnosing depression including face-to-face structured clinical interview, assessment tools and the criteria of DSM-5 (Rooney et al., 2011). One of the most frequently used assessment tool to understand patients' severity of depression level is the Beck Depression Inventory-II (Archer et al., 1991; Beck et al.,1996). In this psychological testing instrument, there are 21 items that individuals are asked to rate on a 4-point scale (range from zero to three). The total score can be between zero and 63 (Beck et al., 1996). If this total BDI-II score is in the range between zero and 13, it shows

‘’minimal depression’’, between 14 and 19 is ‘’mild depression’’, between 20 and 28 is ‘’moderate depression’’ and between 29 and 63 is ‘’severe depression’’. (Beck et al., 1996) According to Kendall et al. (1987), BDI scores indicate all range from the absence of pathology to severe depression. However, BDI is not always used to assess the severity of depression in patients but also to screen for possible clinical depression in health individuals (Archer et al., 1991).

Startle reflex is a particularly useful tool for research on fear (Lonsdorf et al., 2017) and to assess emotional reactivity in humans (Bradley et al., 2001). The startle reflex is a skeletomuscular response of many organisms following aversive and intense stimuli with a sudden onset (Cook et al.,1991). The startle response is a defensive sequence of ancestral defensive reflexes shown to a sudden sensory event (Landis & Hunt, 1939) and a behavioural interruption to cope with the possible threat (Lang, 1995). According to Kaviani (2004) and Lissek et al. (2005), startle response is elicited by a strong and sudden sensory stimulus called a burst of loud white noise. The startle response had a short latency twitch of skeletal and facial muscles in response to this intense acoustic stimulus (Koch, 1999) and the most reliable startle reflex accepted in human studies is the eyeblink response (Lissek et al., 2005).

There are many studies about startle response to investigate emotional reactivity in healthy people but only a little research in clinically depressed individuals. Vrana (1988) suggested that healthy or non-depressed participants showed more startle responses to an aversive acoustic stimulus when they saw negative affective slides like snakes compared to pleasant and positive slides (e.g. attractive models). Correspondingly, in the research by Kaviani et al (2004), emotional stimuli as pleasant, neutral and negative clips were shown to low and high depressive individuals with a white aversive sound to elicit startle blink response. The startle response was potentiated in low depressed individuals to negative clips and attenuated to positive ones. On the contrary, high depressed individuals showed a flat startle response to positive, negative and neutral clips meaning no differentiation across the different emotional stimuli. In other words, there is a lack of startle response modulation with unpleasant as well as pleasant clips.

In addition to these results, in another study, participants were shown positive, negative and neutral clips and asked how positive or negative these clips were for them (Kaviani et al., 2004). As a result, while participants with low depression rated negative clips as more negative and positive clips as more positive, positive video clips were rated less positive and negative video clips also less negative by highly depressed participants. Besides, there was no difference in ratings of neutral clips between control group as healthy individuals and people with

depression. These results showed how emotional and physiological responses to stimuli were affected by depression.

Fendth and Koch (2013) stated that the acoustic startle response can be altered or enhanced also by stress. According to Fani et al., (2011), individuals who were exposed stress reported more fear-potentiated startle response to a cue compared to non-stressed people.

Selye (1976) defined stress as ‘’the nonspecific response of the body to any demand made upon it means the rate at which we live at any moment’’ (p. 138). According to him, when humans are under stress, the tear or wear might occur in their bodies. Similarly, Elkin (1999) demonstrated that stress might cause significant changes in medical and physiological conditions. Stress is also regarded as a dynamic condition in which people come up against pressure, constraint or urgency associating with uncertain outcomes (Hughes et al., 2016; Schuler, 1980).

The American Institute of Stress (1999) showed that stress is perceived as a danger for individuals' systems. For this reason, all systems like the immune and digestive system, the heart and blood vessels, brain or sensory organs need to be modified to adapt to stress. The human body reacts to the abnormal circumstances in this way due to the aim of maintaining bodily functions (Sorenson, 2007). For instance, Maniam and Morris (2014) suggested that stress can activate the hypothalamic–pituitary–adrenal (HPA) axis modulating the degree of adaptation and response. It is shown that stress increases the heart rate (Notarius & Levenson, 1979) and causes to have high blood pressure (Sorenson, 2007). Besides, as a response to stress, our body increases the secretion corticotropin (ACTH) and cortisol, growth hormone, catecholamines, beta-endorphin and prolactin (Berk et al., 1989). At the same time, these hormones are sometimes accepted as stress hormones as well.

According to Wolf (2017), stress can alter not only the physiological response mentioned above but also creates long-lasting effects on individuals' health. According to Stam and colleagues (2000), these long-term changes might be seen in hormonal, autonomic or behavioural responsivity. It is clear that being exposed to stress is a risk factor for the development of long-term depression (LTD) in time (Shors et al., 1989) and also stress creates significant changes on the structure of neurons in the hippocampus, prefrontal cortex, and amygdala (Leuner & Shors, 2013; Vyas A., 2002). As a proof of this, Shors et al. (1989) illustrated that stressors reduce dendritic spine number in the hippocampus. Recent findings of memory and stress indicated that our memory retrieval is influenced by stress as well because hippocampus involved in the memory process. (Andreatta et al., 2015; Bouton, 2004; Bulkin et al., 2016; Klinke et al., 2020; Rudy, 2009; Wolf, 2017). Besides, according to Wolf (2017),

there is a possibility to have long-term consequences owing to the effect of stress on memory process.

Beckers et al. (2013) demonstrated that studies with fear conditioning paradigm give information about fear, learning or memory generally and fear learning specifically as well. The basic procedure of this paradigm includes the match of an initially neutral stimulus (CS or the conditioned stimulus) with an intrinsically aversive stimulus (the US or the unconditioned stimulus) repeatedly. In this way, the CS presentation might entail the physiological or behavioural reactions as an indication of fear (Beckers et al., 2013; Fendt & Fanselow, 1999).

Klinke et al. (2020) investigated the impact of the stress on fear memory. In this research, 30 min before fear conditioning, half of the participants were exposed to Socially Evaluated Cold Pressor Test (SECPT; Schwabe et al., 2008) while the other half were not. During the acquisition phase of this paradigm, one geometrical shape (CS+) was matched with painful electric shock (US). However, another geometrical shape (CS-) presented alone. After both stimuli were presented continuingly, in the next phase called extinction, the two geometrical shapes were presented again but without electric shock (US). In this way, a new or safety memory trace to the CS+ was expected to occur (Bouton, 2004) at the end of the extinction phase. To elicit a physiological response in this study, startle probes were randomly delivered after CS onset in both phases. At the end of the study, it was found that participants who underwent SECPT as a stressor showed startle potentiation to CS+ compared to CS- in both acquisition and extinction phase meaning that extinction learning was not successful in the stress group. As participants exposed to stress and matched the CS+ with the electric shock in the acquisition phase, they still remembered the differentiation between CS+ and CS- at the end of extinction even if there was no shock was presented. It suggested how the impact of stress is strong on fear learning.

A large number of studies clearly stated that stressors (Brown et al., 1985) and stressful life events (Harkness & Luther, 2001; Mazure, 1998) play a major aetiological role for the onset of major depression (Kendler et al., 1998) and also long-term depression (Shors et al., 1989). On the other side, Rudolph et al. (2000) suggested that depression itself or negative thought associated with it might have precipitated the stress reactions. Additionally, studies made clear that depressed people are especially sensitive to stressors which could pave the way for the emergence of major depressive episodes (Anisman & Matheson, 2005; Griffiths et al., 2000) showing the reciprocal impact of stress and depression.

In this present study, as there was insufficient research on non-clinical depressed individuals, I conducted with healthy individuals who have slightly high depressive scores which might be a risk factor for depression (Costa & McCrae, 1992). In addition to this, I investigated the interaction between one stage before the clinical depression level and stress on both verbal and physiological responses. If it is possible to see how stress can be effective on the preclinical depression level, some solutions can be found to prevent to turn this preclinical stage of depression into a clinical depression. Thus, I researched how emotional responses to stress is different for people who have high depressive scores.

In this study, I hypothesized three levels of hypothesis for each phase of fear conditioning.

On the acquisition day, I expected that the ratings to CS+ should be more negative and fearful than the ratings to CS- and startle response should be potentiated to the CS+ as compared to CS-. Additionally, I expected that the ratings to CS+ in the stress group should be more negative and fearful as compared to the control group and startle potentiation to CS+ in the stressed group is potentiated as compared to the control group. I also expected that the fear and valence ratings to CS+ should be more negative and fearful in high depressive participants compared to non-depressive individuals and startle response to CS+ in high depressive individuals should be less potentiated as compared to non-depressive people.

On the extinction day, I expected that the ratings to CS+ should be less negative and fearful at the end of extinction as compared to the end of the acquisition day and startle response to the CS+ should be reduced as compared to startle response to CS+ on the acquisition day. Moreover, I hypothesized that valence and fear ratings to CS+ should be more negative and fearful in the stress group as compared to the control group and there should be startle potentiation to CS+ in the stress group as compared to the non-stress group. I also expected that ratings are more negative and fearful to CS+ in the high depressive individuals as compared to ratings in non-depressive people and startle response to CS+ is stronger in high depressive people as compared to non-depressive individuals.

On the test day, I expected that the ratings to CS+ should be more negative and fearful than the ratings to CS- and startle response should be potentiated to the CS+ as compared to CS-. Moreover, I hypothesized that the ratings to CS+ in the stress group should be more negative and fearful as compared to the control group and startle potentiation to CS+ in the stressed group is stronger as compared to the control group and. I also expect that startle response to CS+ in high depressive participants should be less potentiated as compared to non-depressive people and the valence ratings to CS+ should be stronger in high depressive participants compared to non-depressive individuals.

Methods

Participants

Due to the restrictions related to the outbreak of the virus COVID-19, I had to use existing data collected previously in the University of Würzburg (Germany). The sample consisted of 79 male participants, aged between 18 and 35 years. These participants had no neurological or psychiatric disorders, did not use drugs and did physical exercise less than 10 hours every week. Half of these participants belong to the stress group and the other half were in the control/sham group. The descriptive statistics of ratings and startle responses of the two groups in all phases were shown as tables and figures. From 79 participants, 14 participants were excluded: two of them had mean startle response less than five and therefore defined as non-responder, three of them had missing responses on acquisition and extinction day, two of them had technical problems and seven of them did not have BDI scores. Thus, the final sample consisted of 65 participants.

Material

Unconditioned stimulus (US)

A current stimulator (Digitimer DS7A, Digitimer Ltd., Welwyn Garden City, UK) generated a mildly painful electric stimulus with a frequency of 50 Hz and duration of 200 ms. The intensity of electric shock was individually determined and the initial intensity specified at 0 mA which gradually ascended or descended in 0.5 mA steps. Participants were asked to rate each stimulus on an analogical scale from zero meaning "no sensation at all" to ten meaning "very strong pain" with 4 as a threshold (just noticeable pain). The mean intensity of 1.46 mA (SD = .61) and was rated as painful (M = 5.36, SD = .98).

Conditioned Stimuli (CS)

Two geometrical neutral shapes were used as CSs and their size was 8 cm. These shapes were presented on a black computer screen for 8 s as CS+ and CS- respectively.

Startle Probes

The burst of the white noise of 103 dB with a duration of 50 ms was presented as a startle probe binaurally via headphones.

Subjective Ratings

After each experimental phase, participants were obliged to rate visual stimuli. After the shapes were presented 1 s, they pressed the buttons on keyboard based on visual analogue scales

(VAS) in a range of between one and nine. The meaning of the one was ‘’negative’’, ‘’calm’’ and ‘’no fear’’ for the valence, arousal and fear ratings respectively; while the meaning of the nine was ‘’positive’’, ‘’intense’’ and ‘’strong fear’’, respectively. Furthermore, participants were asked to rate the contingency between the CS+ and the US ranging from zero ‘’no association’’ to 100 ‘’perfect association’’, which means whether participants explicitly learned the association between the CS+ and the electric shock. In other words, valence, arousal, fear and US expectancy (contingency) ratings were collected. In this study, only valence and fear ratings were analyzed.

Questionnaires

Participants filled out Beck’s Depression Inventory (BDI; Beck et al., 1996) after stress induction and it showed the participants who have a high level of depressive traits. Additionally, the Positive and Negative Affect Schedule (PANAS, Krohne et al., 1996) was completed as a questionnaire to understand the current mood of participants. In this study, only BDI scores were analyzed.

In addition to PANAS, arousal and US expectancy ratings, cortisol level of participants was collected but not analyzed as well. As a consequence, no further information about these two ratings, PANAS and cortisol level will be given in this study.

Procedure

Participants came to the laboratory in the afternoon at the same time on four consecutive days.

Stress/ Control Protocols

After their cortisol level was measured, the stress protocol called Socially Evaluated Cold Pressor Test (SECPT) started (Schwabe et al., 2008). In this test, participants (N = 35) had to immerse their dominant hand in cold water (0-2 °C) for a maximum of three minutes. At the same time, the experimenter looked at them seriously without talking and their emotional responses were videotaped. On the other hand, participants in the control group (N = 30) put their dominant hand in lukewarm water (27°C) for a maximum of three minutes as well. Contemporaneously, the experimenter did not look at the participants and their emotional responses were not videotaped.

On the first day, the informed consent was first signed. After their cortisol level was measured, the stress or control protocol was implemented according to the group difference. After this stress exposure, the experimenter waited 30 min to measure the peak of cortisol.

During these 30 minutes, participants filled the BDI. In addition to BDI, the PANAS was used as a questionnaire to measure the current mood of participants. After the application of SECPT and measurement of cortisol level, the electrode was attached to the participants and the calibration of the electric stimulation as an unconditioned stimulus (US) was applied. Participants rated how painful the electric shock was. After these procedures, participants left and they came back to the laboratory 24 hours later.

At the beginning of the second day of the experiment, the cortisol level of participants was measured again, PANAS was filled by the participants and they were asked how painful the electric shock was. Afterwards, the habituation phase started. During this phase, there was no startle probe or shock was delivered. The two different geometrical shapes were presented to all participants. Each picture was presented two times for 8 seconds with an inter-trial interval (ITI) lasting between 15 sec and 20 sec. Afterwards, individuals were asked how positive or negative these pictures were for them to measure valence ratings, how exciting were the pictures for them to measure arousal ratings and how strong was their fear for this picture to measure fear ratings. These ratings worked as baseline ratings and the very same three questions were asked before and after each phase.

After the habituation phase, during an acquisition phase, one shape was associated with the electric shock (i.e. the unconditioned stimulus, US) and then labelled conditioned stimulus (CS+ or threatening signal), but never the other shape (CS- or safety signal). Each shape was shown 16 times and the ITI lasted again 15-20 sec. During half of the CS trials (i.e. eight times) as well as during the ITI, white noises (103 dB, 50 ms) were presented to elicit the startle response. So that at the end of this procedure, we could have eight startle response to CS+, eight to CS- and eight during ITI. At the end of the acquisition phase, valence, arousal and fear ratings for both CS+ and CS- were asked again, as well as US expectancy. In this phase, it was expected that participants rated CS+ and CS- similarly around 5 (neutral) out of 10 in the analogical scale because both shapes of CS+ and CS- were neutral and kind of equal.

After 24 hours, participants came back to the laboratory and before the phase began, individuals were asked to rate the valence, arousal and fear to CS- and CS+, US expectancy and their cortisol level was measured. The extinction phase consisted of two identical blocks. During each extinction block, CS- and CS+ were shown 12 times each without the US and for half of the trials (i.e. 6 times out of 12), the white noise was presented as well as six times during the ITI. After each extinction block, participants were asked to rate valence, arousal and fear of the two shapes. Additionally, at the end of extinction block 2, participants were asked

to rate US expectancy. As usual, their cortisol level and the PANAS were collected at the end of this experimental day.

Two weeks later, participants came to the laboratory for the test phase. Before this last phase, to verify the return of fear, spontaneous recovery which is known as renewal effect (Bouton &Bolles, 1979) was measured by the comparison between the last two trials of the ratings, startle response at the end of the extinction day and the first two test trials of the ratings, startle response at the beginning of the test day. Thus, the recovery of the extinguished fear responses was measured to see the impact of the fear on the learning.

At the beginning of the test day, participants' cortisol level as well as their current mood were checked. In order to verify what they remember, they were then asked to rate valence, arousal and fear of CS- and CS+ as well as US expectancy. CS- and CS+ were shown 16 times each without electric shock as a re-extinction procedure. During 8 times in each, white noise was delivered during both geometrical shapes as well as during the ITI. As a result, I have eight startle responses to CS+, eight startle responses to CS- and eight startle responses to ITI. At the end of this phase, the valence, arousal and fear ratings to both CS- and CS+, US expectancy, the cortisol level and current mood were measured.

Data Reduction

Startle responses as physiological responses were recorded with a V-Amp 16 amplifier and Vision Recorder Software (Version 1.03.0004, BrainProducts Inc., Munich Germany). A notch-filter at 50 Hz and a sampling rate of 1000 Hz were applied. The Brain Vision Analyzer (Version 2.0, BrainProducts Inc., Munich, Germany) was used for offline analyses. Besides, the startle response as a physiological response was measured with an electromyogram (EMG).

Statistical Analysis

The SPSS software (Version 20.0, SPSS Inc.) was used for the statistical analysis. The significance level was accepted as $p < .05$ and the marginal level was set up $.05 < p < .07$ for the statistics of this study. At the beginning and end of all phases (acquisition, extinction and test), repeated-measures analyses of variance (ANOVA's) were applied separately with groups (stress and control) as a between-subjects factor, stimulus (CS+ and CS-) as a within-subjects factor, ratings and startle response as outcome variables. Additionally, BDI scores were used as a covariate in the Analysis of Covariance (ANCOVA) and they were measured for stress and control group separately as well. To investigate the return of fear, ANOVA was calculated with the last two trials of extinction ratings, startle response and the first two trials of extinction ratings, startle response of the test phase. Similarly, depressive scores were accepted as a

covariate in the ANCOVA for spontaneous recovery as well. To verify whether the stress or the control group differences in depression in general, a t-test was performed between stress and control group regarding BDI total scores. If the assumption of sphericity was violated, the Greenhouse-Geisser correction (GG-ε) of the degree of freedom was used in the statistical analysis. For post hoc tests, simple contrasts were performed as follow-up analyses and Bonferroni corrected.

Results

For acquisition day, the ANOVA for valence ratings showed a significant main effect of time, $F(1, 63) = 17.91$, $p < .001$, $\eta_p^2 = .221$ meaning that the valence ratings significantly changed from before and after acquisition independently from CS+ and CS-. Likewise, there was a significant main effect of stimulus, $F(1,63) = 17.82$, $p < .001$, $\eta_p^2 = .221$, stated that CS+ was rated more negative as compared to CS- independently from time. As their interaction was also significant, $F(1,63) = 12.94$, $p = .001$, $\eta_p^2 = .170$, indicating that CS+ and CS- were rated differently before and after acquisition phase (see Figure 1). Because of this interaction, post hoc analysis was conducted (Bonferroni correction: $p = .025$). According to results, CS+ and CS- were rated with equal valence before the acquisition phase, $t(64) = 1.13$, $p = .265$, while the valence ratings of CS+ was more negative than CS- at the end of acquisition, $t(64) = 5.64$, $p < .001$ (see Table 1). Similarly, the ANOVA for fear ratings revealed a significant main effect of time, $F(1, 63) = 64.26$, $p < .001$, $\eta_p^2 = .505$, meaning that the fear ratings significantly changed from before and after acquisition independently from CS+ and CS-. Likewise, there was a significant main effect of stimulus, $F(1,63) = 18.10$, $p < .001$, $\eta_p^2 = .223$, indicated that CS+ was rated more threatening or fearful as compared to CS- independently from time. Since their interaction was also significant, $F(1,63) = 13.29$, $p = .001$, $\eta_p^2 = .170$, it suggested that CS+ and CS- had different fearful ratings in before and after acquisition phase (see Figure 2). To see where the ratings changed, post hoc analysis was conducted (Bonferroni correction: $p = .025$). According to the results, CS+ and CS- were rated with equal fear before the acquisition phase, $t(64) = 0.77$, $p = .444$, while the fear ratings of CS+ was more fearful than CS- at the end of acquisition, $t(64) = 6.30$, $p < .001$ (see Table 2). In terms of startle response, even if there was startle potentiation to CS+, this difference was not noticeable because there was no significant effect for stimulus, $F(1, 63) = .36$, $p = .551$, $\eta_p^2 = .006$), showing that there was no difference in startle response to CS+ and CS- (see Table 3).

There was no significant main effect for the factor group on the valence ratings in the acquisition (all ps $> .442$). Regarding to group, no significant differences were found in

participants' fear ratings in acquisition (all *p*s > .262). To conclude, these results stated that regarding valence and fear ratings, being in stress or control group did not play a significant role in the valence and fear ratings to CS+ and CS-. In addition, there was also no significant main effect of factor group on startle response, $F(1, 63) = 1.16$, $p = .286$, $\eta_p^2 = .018$, as well as the interaction between stimulus and group, $F(1, 63) = .03$, $p = .867$, $\eta_p^2 < .001$. As a result, there was no difference in the startle response to CS+ and CS- in stress or control group in the acquisition phase (see Table 1). The ANCOVA for BDI scores showed no significant effect on valence ratings in the stress group and control group (all *p* values >.242).There was found only marginal effect of BDI with time in the control group, $F(1,28) = 3.85$, $p = .060$, $\eta_p^2 = .121$. The effect of high depressive scores on fear ratings in the acquisition had similar results. Neither main effect nor the interaction effect had a significant effect in both stress and control group (all *ps* > .115).In terms of startle response, depressive scores revealed no significant main effect and also interaction with the stimulus in both stress and control group (all *p*s > .162). These results revealed that BDI scores did not influence the acquisition learning on the valence and fear ratings, as well as startle response to CS+ and CS-.

Table 1

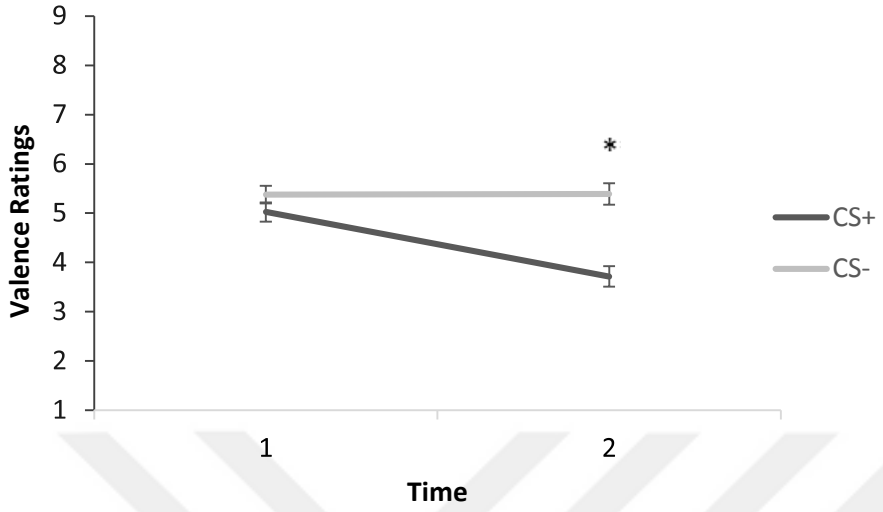
Descriptive Statistics of Valence Ratings on Acquisition Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Acq	5.11	1.49	4.93	1.64
CS Min Pre Acq	5.26	1.32	5.5	1.55
CS Plus Post Acq	3.6	1.4	3.83	1.91
CS Min Post Acq	5.34	1.61	5.43	1.91

Note. The univariate analysis of variance is performed to find out the significant differences between stress ($N = 35$) and control group ($N = 30$), ($p < .05$).

Figure 1

Paired-samples Test of Valence Ratings on Acquisition Day



* p < .025

Table 2

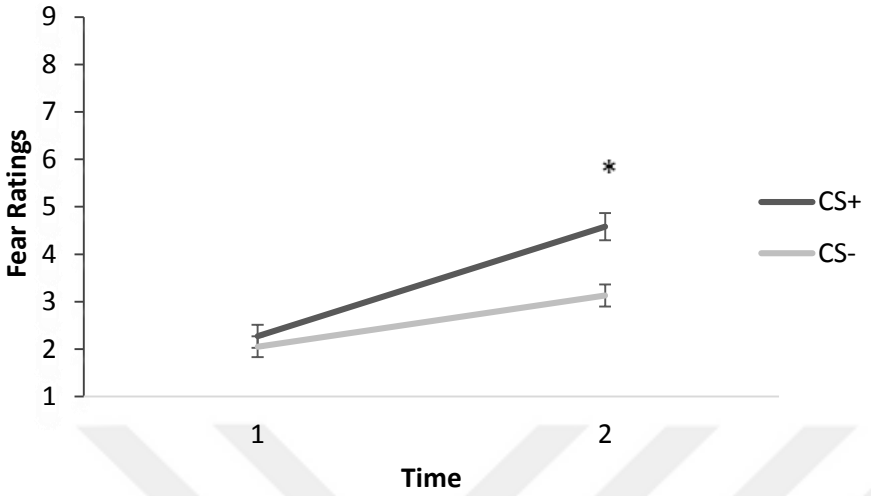
Descriptive Statistics of Fear Ratings on Acquisition Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Acq	2.37	1.82	2.17	2.12
CS Min Pre Acq	2.17	1.82	1.93	1.68
CS Plus Post Acq	5.06	2.34	4.1	2.28
CS Min Post Acq	3.26	1.88	3	1.89

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 35) and control group (N = 30), (p < .05).

Figure 2

Paired-samples Test of Fear Ratings on Acquisition Day



* p < .025

Table 3

Descriptive Statistics of Startle Response on Acquisition Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Acq	7.31	11.65	5.14	6.30
CS Min Acq	6.80	12.81	4.22	7.16

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 35) and control group (N = 30), (p < .05).

During the extinction phase, the ANOVA for valence ratings revealed not significant main effect of time, $F(1.62, 102.34) = 0.46$, GG- $\varepsilon = 0.812$, $p = .594$, $\eta_p^2 = .007$, meaning that in terms of the valence ratings, there was no noticable change among pre, block 1 and post extinction phase. In contrast, there was statistically significant effect of stimulus, $F(1,63) = 18.70$, $p < .001$, $\eta_p^2 = .229$, as well as the interaction between time and stimulus resulted significant, $F(1.52, 85.87) = 6.95$, GG-$\varepsilon = 0.76$, $p = .004$, $\eta_p^2 = .099$. These results showed that CS+ was rated more negatively compared to CS- during the 3 time points of extinction phase.

Following the significant interaction, post hoc analysis was conducted (Bonferroni correction: $p = .017$). Results indicated that CS+ was rated more negative than CS- in pre-extinction, $t(64) = 4.86$, $p < .001$, block 1, $t(64) = 2.81$, $p = .007$, and post extinction, $t(64) = 2.54$, $p = .014$ respectively. As CS+ was rated still more negative than CS- at the end of extinction phase, it resulted that extinction learning was not successful for valence ratings (see Table 4). Similarly, the ANOVA for fear ratings revealed not significant main effect of time, $F(1.68, 105.75) = 2.80$, GG-$\varepsilon = 0.84$, $p = .074$, $\eta_p^2 = .043$ meaning that in terms of the fear ratings, there was no noticable change among pre-extinction, block 1 and post extinction phase. In contrast, there was a statistically significant effect of stimulus, $F(1,63) = 28.58$, $p < .001$, $\eta_p^2 = .312$, as well as the interaction between time and stimulus resulted significant, $F(1.61, 101.17) = 31.19$, GG-$\varepsilon = 0.80$, $p < .001$, $\eta_p^2 = .331$. According to post hoc analysis results (Bonferroni correction: $p = .017$), CS+ was rated more fearful compared to CS- at the beginning of the extinction phase, $t(64) = 7.24$, $p < .001$ and in the block 1, $t(64) = 3.35$, $p = .001$ respectively. In contrast, at the end of the extinction, CS+ and CS- were rated equal valence at the end of extinction, $t(64) = 1.40$, $p = .165$ (see Table 5). In terms of startle response, in the ANOVA, the interaction between time and stimulus has no significant but marginal effect, $F(1, 63) = 7.80$, $p = .061$, $\eta_p^2 = .110$. Similarly, no significant main effects of startle response were found for time and stimulus (all ps > .580). These results illustrated that there was no difference between the startle response to CS+ and CS- (see Table 6).

There was no significant effect for the factor group on the valence ratings in the extinction (all ps > .234) except the marginal effect of time and group interaction, $F(2,126) = 3.47$, GG-$\varepsilon = .812$, $p = .067$, $\eta_p^2 = .052$. In contrast to valence ratings, there was a significant effect of interaction between time, stimulus and group interaction on participants' fear ratings, $F(1.61,101.17) = 6.06$, GG-$\varepsilon = 0.803$, $p = .006$, $\eta_p^2 = 0.088$, however no other effect involving the factor group were found (all ps > .424). To see how CS+ and CS- were rated differently in the two groups during extinction, post hoc analysis was conducted (Bonferroni correction: $p = .017$). Results suggested that both stressed and non-stressed people rated CS+ more fearful compared to CS- at the beginning of extinction respectively. In block 1, similarly, stressed participants had more fearful ratings to CS+ compared to CS- whereas, in the control group, there was no difference in fear ratings for CS+ and CS- in block 1. At the end of extinction, participants who exposed to stress still rated CS+ more threatening than CS- (see Figure 3), however non-stressed individuals rated fear to CS+ and CS- equal (see Figure 4). It stated that in terms of fear ratings, there was impaired extinction learning in the stress group but the non-stressed group showed successful extinction learning at the end of this phase. Moreover, there

was no significant main effect and the interaction effects involving factor group on startle response (all *p*s > .166). It showed that stressed and non-stressed participants had similar startle response to CS+ and CS-. (See Table 3). The ANCOVA for BDI scores showed no significant effect including the main effect on valence ratings in the stress group and control group (all *p*s >.102).There was found no significant but only marginal effect of BDI with time in the control group, $F(1.88, 52.74) = 3.44$, GG-$\varepsilon = 0.942$, $p = .058$, $\eta_p^2 = .109$, in this phase. In terms of fear ratings, there was a significant effect of the time and BDI interaction in the stress group, $F(1.49, 49.18) = 3.63$, GG-$\varepsilon = 0.745$, $p = .046$, $\eta_p^2 = 0.099$, whereas no other significant effect found for the depression scores on the fear ratings in the extinction. Because of this result, post hoc correlation for the interaction between time and BDI interaction was conducted. The results suggested that in the stress group, fear ratings at the end of extinction was positively correlated with depressive scores (Pearson's $r(35) = .39$, $p < .01$). On the contrary, there was no significant effect of BDI on the startle response to CS+ and CS- including the main effect in both control and stress group (all *p*s >.12).

Table 4

Descriptive Statistics of Valence Ratings on Extinction Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Ext	4.29	1.20	4.13	1.74
CS Min Pre Ext	5.37	1.61	5.8	1.79
CS Plus Block 1	4.71	1.47	4.3	1.62
CS Min Block 1	5.37	1.57	5	1.74
CS Plus Post Ext	4.86	1.38	4.57	1.59
CS Min Post Ext	5.49	1.48	4.93	1.84

Note. The univariate analysis of variance is performed to find out the significant differences between stress ($N = 35$) and control group ($N = 30$), ($p < .05$).

Table 5

Descriptive Statistics of Fear Ratings on Extinction Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Ext	3.97	2.07	4.67	2.31
CS Min Pre Ext	2.4	1.54	2.5	1.98
CS Plus Block 1	3.66	1.88	3.43	2.24
CS Min Block 1	2.74	1.74	3.13	2.21
CS Plus Post Ext	3.2	2.04	3.07	1.98
CS Min Post Ext	2.57	1.79	3.23	2.3

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 35) and control group (N = 30), ($p < .05$).

Table 6

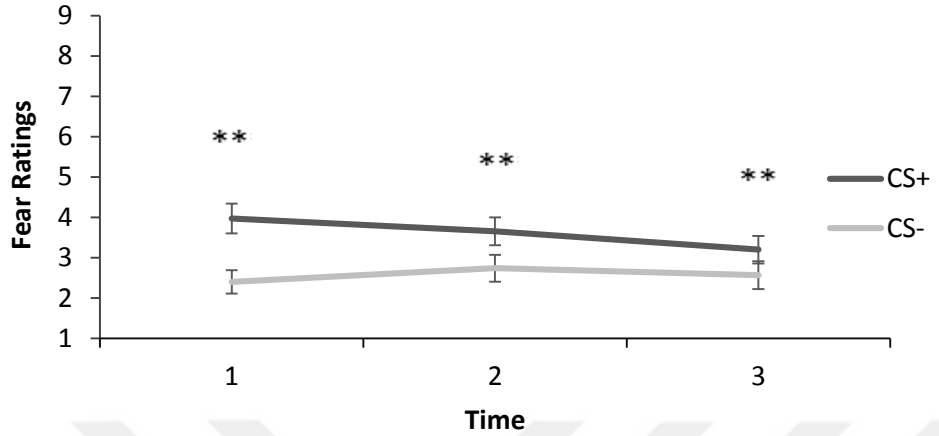
Descriptive Statistics of Startle Response on Extinction Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus First Block	6.93	15.62	4.78	8.91
CS Min First Block	5.66	12.74	0.21	7.94
CS Plus Second Block	3.90	9.27	4.15	8.90
CS Min Second Block	7.65	12.9	4.99	9.19

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 35) and control group (N = 30), ($p < .05$).

Figure 3

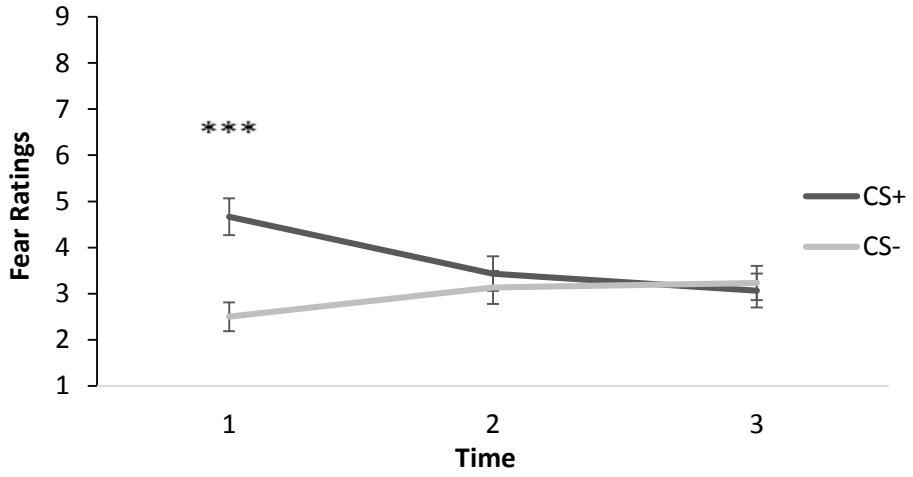
Paired-samples Test of Fear Ratings in Stress Group on Extinction Day



** p < .017

Figure 4

Paired-samples Test of Fear Ratings of Control Group on Extinction Day



*** p < .01.

The ANOVA for the comparison between at the end of extinction and at the beginning of the test to verify the return of fear, valence ratings suggested that there was a main effect of stimulus, $F(1, 57) = 15.85$, $p < .001$, $\eta_p^2 = 0.218$, as well as time and stimulus interaction, $F(1,57) = 8.19$, $p = .006$, $\eta_p^2 = 0.126$. To clarify this interaction, post hoc analysis was conducted

(Bonferroni correction: *p*). The ANOVA for the comparison between at the end of extinction and at the beginning of the test to verify the return of fear, valence ratings suggested that there was a main effect of stimulus, $F(1, 57) = 15.85$, $p < .001$, $\eta_p^2 = 0.218$, as well as interaction between time and stimulus, $F(1,57) = 8.19$, $p = .006$, $\eta_p^2 = 0.126$. To clarify this interaction, post hoc analysis was conducted (Bonferroni correction: $p = .025$). According to results, CS+ were rated more negative than CS- at the end of extinction, $t(64) = 2.54$, $p = .014$ and the beginning of the test, $t(58) = 4.27$, $p < .001$) respectively (see Table 7). However, there was no significant effect was found for the time, $F(1, 57) = 0.36$, $p = .509$, $\eta_p^2 = .478$. There was a significant main effect for stimulus, $F(1, 57) = 3.68$, $p = .008$, $\eta_p^2 = 0.115$, on fear ratings showing that participants rated CS+ more fearful than CS- independently from the time. The interaction between time and stimulus showed a marginal effect rather than significant effect, $F(1, 57) = 7.43$, $p = .060$, $\eta_p^2 = 0.061$. However, there was no significant effect for the time on fear ratings, $F(1, 57) = 0.01$, $p = .933$, $\eta_p^2 = .000$ (see Table 8). The ANOVA for startle response suggested that there was no main effect of time, $F(1, 55) = 2.81$, $p = .100$, $\eta_p^2 = 0.049$, as well as stimulus, $F(1, 55) = 1.11$, $p = .298$, $\eta_p^2 = 0.020$. However, interaction between time and stimulus resulted the significant effect on startle response, $F(1,55) = 7.45$, $p = .009$, $\eta_p^2 = 0.119$. Post hoc tests suggested that at the end of the extinction, startle response to CS+ was less than CS- whereas at the beginning of the test phase, startle responses were potentiated to CS+ as compared to CS- (see Table 9).

There was no significant effect for the group including the main effect on valence ratings in this comparison between the end of extinction and the beginning (all *p*s >.123), similarly on fear ratings (all *p*s >.139) and the startle responses (all *p*s >.156). The ANCOVA for BDI scores showed no significant effect including the main effect on valence ratings in the stress group and control group (all *p*s >.195) on the spontaneous recovery. For fear ratings, time and BDI interaction has a significant effect on the stress group, $F(1, 28) = 4.99$, $p = .034$, $\eta_p^2 = .151$, whereas no further significant effect was found for BDI scores except this interaction (all *p*s >.076). Because of this result, post hoc correlation for the time and BDI interaction was conducted. According to the correlation results, it is revealed that in the stress group, fear ratings at the end of extinction is positively correlated with depressive scores (Pearson's $r(35) = .39$, $p = .022$). The ANCOVA for startle response suggested that there were no significant effects including the main effect of BDI in both stress and control group (all *p*s >.356).

Table 7

Descriptive Statistics of Valence Ratings on Spontaneous Recovery

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Post Ext	4.77	1.36	4.62	1.59
CS Min Post Ext	5.27	1.41	5	1.83
CS Plus Pre Test	4.37	1.03	4.07	1.31
CS Min Pre Test	5.2	1.24	5.59	1.78

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 30) and control group (N = 29), ($p < .05$).

Table 8

Descriptive Statistics of Fear Ratings on Spontaneous Recovery

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Post Ext	3.23	2.06	3.07	2.02
CS Min Post Ext	2.67	1.81	3.14	2.28
CS Plus Pre Test	3.7	2.15	3.14	2.20
CS Min Pre Test	2.93	1.82	2.41	1.88

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 30) and control group (N = 29), ($p < .05$).

Table 9

Descriptive Statistics of Startle Response on Spontaneous Recovery

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Post Ext	-0.57	13.17	-1.81	13.88
CS Min Post Ext	2.23	16.58	1.15	9.33
CS Plus Pre Test	11.61	25.06	3.42	14.02
CS Min Pre Test	2.72	20.95	1.41	15.28

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 29) and control group (N = 28), ($p < .05$).

The ANOVA for the test day indicated that significant main effect of stimulus, $F(1, 57) = 22.40$, $p < .001$, $\eta_p^2 = 0.282$, as well as Time x Stimulus interaction, $F(1, 57) = 4.05$, $p = .049$, $\eta_p^2 = 0.066$, for valence ratings. Post hoc contrasts (Bonferroni correction: $p = .025$) revealed that participants rated CS+ more negative than CS- at the beginning of test, $t(58) = 4.27$, $p < .001$and at the end of the test, $t(58) = 4.01$, $p < .001$, respectively (see Table 10). In terms of fear ratings on the test phase, there was found no significance different on time, $F(1, 57) = 1.80$, $p = .185$, $\eta_p^2 = 0.031$, as well as interaction between time and stimulus, $F(1, 57) = .01$, $p = .939$, $\eta_p^2 = 0.00$. However, there was a significant main effect of stimulus, $F(1, 57) = 14.53$, $p < .001$, $\eta_p^2 = 0.203$, proving that CS+ was rated more fearful than CS- independently from time on the test day (see Table 11). There was no significant effect of startle response was found for the stimulus, $F(1, 55) = 1.357$, $p = .249$, $\eta_p^2 = .024$, showing that even if there is a slightly startle potentiation to CS+, this was not a significant effect. For this reason, it was suggested there is no difference in startle response to CS+ and CS- (see Table 12).

Regarding to factor group, no significant effects including the main effect were found in participants' valence ratings (all ps > .225), fear ratings (all ps > .384), as well as startle response (all ps > .415) in the test phase. The ANCOVA of depressive scores for valence ratings was not statistically significant on both stress and control group, all ps > .245. In fear ratings, there was a marginal effect on the Time x BDI interaction in stress group, $F(1, 27) = .72$, $p = .069$, $\eta_p^2 = .026$), but no significant effect in other valence ratings in both stress and control

group (all *p*s > .245). Similarly, no further effect was found including the depressive score on startle response in the test phase (all *p*s > .356) showing that depressed and non-depressed people had equal startle response to CS+ and CS-.

Table 10

Descriptive Statistics of Valence Ratings on Test Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Test	4.37	1.03	4.07	1.31
CS Min Pre Test	5.2	1.24	5.59	1.78
CS Plus Post Test	4.67	1.18	4.41	1.40
CS Min Post Test	5.23	1.57	5.28	1.60

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 30) and control group (N = 29), (p < .05).

Table 11

Descriptive Statistics of Fear Ratings on Test Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Pre Test	3.7	2.15	3.14	2.20
CS Min Pre Test	2.93	1.82	2.41	1.88
CS Plus Post Test	3.33	2.28	2.97	2.15
CS Min Post Test	2.47	1.87	2.38	1.92

Note. The univariate analysis of variance is performed to find out the significant differences between stress (N = 30) and control group (N = 29), (p < .05).

Table 12

Descriptive Statistics of Startle Response on Test Day

Stimuli	Stress		Control	
	M	*SD*	*M*	*SD*
CS Plus Test	4.27	11.22	2.19	9.51
CS Min Test	2.60	9.58	0.94	8.78

Note. The univariate analysis of variance is performed to find out the significant differences between stress ($N = 29$) and control group ($N = 28$), ($p < .05$).

Discussion

In this study, I aimed at investigating how emotional responses to stress might be different for healthy people who have high scores on the BDI. To do so, participants underwent the Socially Evaluated Cold Pressure Test (SECPT) either in a stress group or a control group and also their scores on the BDI were measured.

I expected to see more negative and fearful ratings to CS+ compared to CS- as a general hypothesis in the acquisition phase. The hypothesis was confirmed because, at the end of acquisition, CS+ was rated more fearful and negative than CS-. This result suggested that there was some kind of conditioned responses to CS+ including more fear and more aversive responses due to given shock with CS+ as a US and acquisition learning was successful for verbal ratings in general. These findings on acquisition learning are in line with previous studies as well (Andreatta et al., 2010; Sjouwerman et al., 2015).

In previous studies, there was startle potentiation to CS+ in comparison to CS- in the acquisition phase (Andreatta et al., 2010; Klinke et al., 2020; Lonsdorf, 2015). In this study, there was startle potentiation to CS+ vs. CS- as previous studies but this difference between CS+ and CS- did not reach the significance level in the acquisition phase in general. As this reaction is an automatic response when faced with a threat (Hamm, Weike 2015), the threat could be weak or there was not enough time for being exposed to stress. Thus, it might not have revealed startle potentiation to CS+. Similarly, to increase the effectiveness of startle potentiation, white noise might be given during all of CS trials rather than half of them. Besides, giving stress beforehand, the given white noise could be effective on the startle potentiation as well.

Klinke et al. (2020) suggested that individuals who were stressed 10 days before acquisition phase rated CS+ more negative in comparison to CS- and showed startle potentiation to CS+ vs. CS- in the acquisition phase. Interestingly, there was found to be the statistically non-significant effect of stress in both valence and fear ratings as well as startle response in this study which is not in line with my hypothesis and previous research. One possible reason was the stress was too mild or weak to create differentiation with the control group on ratings and startle response or the time of being exposed was not enough to create stress. Moreover, there might have been cognitive responses to valence and fear ratings due to self-report bias (Lonsdorf et al., 2017) or cognitive responses. As they might have been aware of the expected answers, the tendency to give the same answers might have been important for both groups independently from being exposed to stress or not.

In terms of BDI scores, I hypothesized that valence and fear ratings to CS+ should be stronger in high depressive individuals compared to non-depressive participants and startle response to CS+ in high depressive individuals should be less potentiated as compared to non-depressive people. Interestingly, as depression did not show a significant difference in both ratings and startle response in this study, it was clear that depression did not modulate the acquisition learning on the valence and fear ratings. The possible reason leading this result might have been all participants were still healthy even if they had high depression scores on BDI. Because of this, they might have given answers which they thought the researcher was expecting from them. For instance, high depressed individuals might have presented themselves as low depressed which could affect the results. The possibility of ratings might have been not as sensitive for intraindividual differences.

The results of ratings on extinction day showed a difference for valence and fear ratings. As participants were not exposed to the US anymore, a new memory trace might be formed to CS+ in this phase. According to hypothesis, it was expected to have less negative and fearful ratings to CS+ than CS- and reduced startle response to CS+ vs. CS- in the extinction phase compared to the end of acquisition in the hypothesis. Similarly, in previous studies illustrated that there was no differentiation on valence and fear ratings to CS+ and CS- (Andreatta & Pauli, 2015; Golkar & Ohman, 2012; Klinke et al., 2020; Sjouwerman et al., 2015; Steinfurth et al., 2014). The results demonstrated that participants had more negative valence ratings to CS+ compared to CS- at the end of extinction independently from depression and stress. It showed that they still remember this difference between CS+ and CS- from the day before in the acquisition phase and extinction learning was impaired for valence ratings as compared to acquisition learning. On the contrary, extinction learning was successful for fear ratings, which

is in line with previous research (Golkar & Ohman, 2012; Sjouwerman et al., 2015; Steinfurth et al., 2014). However, this learning was a little bit slow. This might be because there was still more fearful ratings to CS+ compared to CS- in block 1. For this reason, participants might have needed a higher amount of trials to have more extinction learning for valence ratings in general. The reason why there was a successful extinction in fear ratings but not in valence could be related to the difference of nature of fear and valence. If people were not exposed to threatening stimulus, their fear ratings could decrease because there was not any threat anymore. However, if people did not like something or found an object as negative, there was no reason to change their mind and they will probably continue to do not like the same object. Moreover, at the beginning of extinction day, there was found a difference between CS+ and CS-, whereas CS+ and CS- were rated similar in terms of startle response at the end but this effect was marginal, not significant. These results suggested that participants learnt this fear to CS+ on the physiological level but it took a bit longer. Besides, it was clear that there was a successful extinction for startle response meaning that this result was kind of in line with the hypothesis which suggested that at the end of extinction, the startle potentiation would decrease.

Additionally, according to the hypothesis suggesting that ratings to CS+ should be more negative and fearful in the stress group as compared to the control group and there should be startle potentiation to CS+ in the stress group as compared to the non-stress group. It is noteworthy that stress played a significant role in the fear ratings but not in valence ratings in the extinction phase. The results demonstrated that at the end of extinction, participants who exposed to stress still rated CS+ more threatening than CS-, however non-stressed individuals rated fear to CS+ and CS- equal. Stressed individuals showed impaired extinction learning in terms of fear ratings showing that the effect of the electric shock did not fade away compared to the control group. It might have been because they learnt that CS+ was rated more fearful in the acquisition. As participants from the control group were not exposed to stress, they extinguished the fear and maintained this extinction at the end of the extinction phase. Similarly, there was no significant difference in valence ratings in both stress and control group. In contrast to this result, according to Klinke et al. (2020), individuals who were stressed 10 days before extinction showed impaired extinction learning on valance ratings, which shows the difference between the result and the previous study. Moreover, the reason for this difference between fear and valence ratings might have stemmed from the sensitivity of fear ratings. As participants were asked about fear of the electric shock, it might have been more sensitive or emotional than asking about the valence of the stimuli.

Similarly, BDI did not influence the extinction learning on the valence ratings as well as startle response significantly due to possible reasons explained above. In contrast, the more depressive participants were, the more fear ratings they indicated independently from CS+ and CS- at the end of the extinction. This result meant that even if the US was not presented, the combination of depression and stress might have made kind of stronger the fear level of stimuli. The possible reason for the significant effect of depression on fear ratings might be related to rumination (Joos, 2011) as a way of thinking typifies depression (eg. Teasdale & Barnard, 1993). According to Joos (2011), repeatedly thinking (rumination) about the conditioning research might affect the fear reactions. Because of this, depression has a significant effect on fear ratings. As fear ratings were sensitive, it could be triggered to reveal more threatening responses. Moreover, depressed participants who were also exposed to stress might have been more anxious than non-depressed people at the beginning of the extinction because they were predicting to receive the shock as a robust phenomenon (Beckers et al., 2013). As they did not receive this aversive US, this process might be more ambiguous for them. In brief, it was already known that stress before the fear of learning can consolidate the fear memory trace (Rodrigues et al., 2009). Adding also having a high depressive score, expecting to be exposed to the shock and being in the stress group might have induced stronger fear in participants as well. Besides, these results illustrated that depression could mediate the extinction learning more than acquisition because of this significant interaction between time and depression. To sum up, it seems that depression did not really affect the discrimination of CS+ and CS- but it seems that played a role mainly in the extinction independently from the conditions.

The ratings and startle response were examined to see whether there was a spontaneous recovery or not. Due to the difference of the extinction learning of two groups and to verify the returned fear, the ratings and startle response in both groups at the end of the extinction and the beginning of the test were calculated. As participants continued to rate CS+ more negative compared to CS- in both at the end of extinction and the beginning of the test, it was seen that they learnt this result from acquisition phase and it was more difficult to extinguish the difference between the CS+ and CS- valence ratings than the fear ratings. Pavlov (1927) suggested that extinguished conditioned responses would spontaneously recover in time because extinction might inhibit the conditioned response but cannot efface it. Another reason for this result might be related to the nature of the CS+. Even if participants were not exposed to the US anymore in the extinction, the effect and nature of CS+ remained. As valence ratings related to how much they like it, the answers were always the same in all conditions. For fear ratings, it was clearly seen that CS+ was more fearful than CS- in general independently from

time. It was expected because participants had the same results in the acquisition as well as in the extinction. Besides, the more threatening ratings to CS+ compared to CS- of stressed people supported these fear results too. As stress group had impaired extinction learning whereas the control group not, it was seen that time did not play a significant role. Moreover, the reason for being exposed to stress did not play a significant role in both valence and fear ratings might have been less severity of stressor. In terms of startle response, at the beginning of the test, there was startle potentiation to CS+ vs CS-, which is in line with the hypothesis as well as previous studies (Pavlov, 1922).

Not surprisingly, depressed people who were exposed to stress had more fear ratings in the verification of spontaneous recovery as well. These results might have shown they still remember the fear due to its sensitivity and combination with high depressive scores. In the test, the same result was still valid but it had only marginal effect rather than significant effect showing that the interaction between the time and BDI was not effective like another phase due to the kind of re-extinction called test phase. As there was no shock since the acquisition phase and already two weeks passed after extinction, depressive scores did not have any significant role in both valence and fear ratings. Additionally, as little research has been conducted in fear conditioning with non-clinical depressed people, it is not clear these results are in line with previous studies or not.

In the test phase as a re-extinction process, even if participants were not exposed to the US, they continued to rate CS+ more negative than CS- in general, and also before and after the test phase. It illustrated that valence ratings were not extinguished and as it was mentioned above, this result might be related to the nature of the CS+. For fear ratings, participants continued to rate CS+ more fearful in general independently from time. This result illustrates that even if two weeks passed, they still remember that CS+ was linked with stress which means fear learning took place at the end of the test phase. Additionally, stress did not play a significant role in both verbal ratings due to the possible reason mentioned above. On the other hand, there was slightly startle potentiation to CS+ compared to CS- which was expected, however this result did not reach statistical significance. To conclude, it was seen that the test phase did not work as the extinction phase in terms of the startle response. Similar to previous results in other phases, depression did not have a significant impact on ratings as well as startle response owing to probable reasons mentioned above.

Limitations and Future Directions

Being exposed to stress did not have any significant effect on startle response. It might be related to the small sample size or using relatively mild stress. Another reason might be calculating the mean scores of startle responses or it can be possible that the statistical power might not have been enough to reveal the significance effect which was expected. Similarly, as the number of participants on the stress and control group was different, that might have prevented to see expected results. Moreover, as depressed people could tend to ruminate about conditioning even if they were non-clinical participants, it might have been effective in the results as well. Additionally, in some phases, depression had a marginal effect on ratings and startle response. However, it did not reach statistical significance.

To summarize, having an equal number of participants in both experimental and control group and using larger sample size can be taken into consideration for future research. Additionally, as rumination has an impact on fear ratings, The Ruminative Response Scale can be applied to individuals as the most used measure of rumination in reply to depressive moods (Lei et al., 2017). In this way, future studies can be conducted with participants who have less severity of depressive rumination, which might increase the possibility of having a significant effect of depression. Besides, as little research has been conducted in fear conditioning with non-clinical depressed people, the significant and marginal effects of depression can reveal some interesting results with future research.

References

Alford, P. D. B. A. & Beck, M. D. A. T. (2014). The Definition of Depression. In *Depression: Causes and Treatment* (pp. 3–11). University of Pennsylvania Press.

American Psychiatric Association. (2013). Anxiety Disorders. In *Diagnostic and statistical m manual of mental disorders* (5th ed.).

Andreatta, M., Leombruni, E., Glotzbach-Schoon, E., Pauli, P., & Mühlberger, A. (2015). Generalization of Contextual Fear in Humans. *Behavior Therapy*, *46*, 583–596. https://doi.org/10.1016/j.beth.2014.12.008

Andreatta, M., Mühlberger, A., Yarali, A., Gerber, B., & Pauli, P. (2010). A rift between implicit and explicit conditioned valence in human pain relief learning. *Proceedings of the Royal Society B: Biological Sciences, 277*(1692), 2411-2416. https://doi.org/10.1098/rspb.2010.0103

Andreatta, M., & Pauli, P. (2015). Appetitive vs. Aversive conditioning in humans. *Frontiers in Behavioral Neuroscience, 9*. doi:10.3389/fnbeh.2015.00128

Anisman, H., & Matheson, K. (2005). Stress, depression, and anhedonia: Caveats concerning animal models. *Neuroscience & Biobehavioral Reviews*, *29*, 525–546. https://doi.org/10.1016/j.neubiorev.2005.03.007

Archer, R. P., Maruish, M., Imhof, E. A., & Piotrowski, C. (1991). Psychological test usage with adolescent clients: 1990 survey findings. *Professional Psychology: Research and Practice, 22*, 247–252.

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. F. (1996). Comparison of Beck Depression Inventories-IA and-II in Psychiatric Outpatients. *Journal of Personality Assessment, 67*, 588–597. https://doi.org/10.1207/s15327752jpa6703_13

Beck, A. T., Steer, R. A,, & Brown, *G.* K. (1996). *Manual for the Beck Depression Inventory-It.* San Antonio, TX: Psychological Corporation.

Beckers, T., Krypotos, A.-M., Boddez, Y., Effting, M., & Kindt, M. (2013). What's wrong with fear conditioning? *Biological Psychology*, *92*, 90–96. https://doi.org/10.1016/j.biopsycho.2011.12.015

Berk, L. S., Tan, S. A., Fry, W. F., Napier, B. J., Lee, J. W., Hubbard, R. W., … Eby, W. C. (1989). Neuroendocrine and Stress Hormone Changes During Mirthful Laughter. *The American Journal of the Medical Sciences, 298*, 390–396. https://doi.org/10.1097/00000441-198912000-00006

Bouton, M. E. (2004). Context and Behavioral Processes in Extinction. *Learning & Memory, 11*, 485–494. https://doi.org/10.1101/lm.78804

Bouton, M. E., & Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learning and Motivation, 10*, 445–466. https://doi.org/10.1016/0023-9690(79)90057-2

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: Defensive and appetitive reactions in picture processing. *Emotion, 1*, 276–298. https://doi.org/10.1037/1528-3542.1.3.276

Brown, G. W., Craig, T. K. J., & Harris, T. O. (1985). Depression: Distress or Disease? Some Epidemiological Considerations. *British Journal of Psychiatry, 147*, 612–622. https://doi.org/10.1192/bjp.147.6.612

Bulkin, D. A., Law, L. M., & Smith, D. M. (2016). Placing memories in context: Hippocampal representations promote retrieval of appropriate memories. *Hippocampus, 26*, 958–971. https://doi.org/10.1002/hipo.22579

Caverzasi E, Pichiecchio A, Poloni GU, et al. *Magnetic resonance spectroscopy in the evaluation of treatment efficacy in unipolar major depressive disorder: a review of the literature.* Functional Neurology. 2012 Jan-Mar; 27:13-22.

Cook, E. W., Hawk, L. W., Davis, T. L., & Stevenson, V. E. (1991). Affective individual differences and startle reflex modulation. *Journal of Abnormal Psychology, 100*, 5–13. https://doi.org/10.1037/0021-843x.100.1.5

Costa, P. T., & Mccrae, R. R. The Revised NEO Personality Inventory (NEO-PI-R). *The SAGE Handbook of Personality Theory and Assessment: Volume 2 — Personality Measurement and Testing*, 179–198. https://doi.org/10.4135/9781849200479.n9

Elkin, A. (1999) *Stress management*. New York:Wiley.

Fani, N., Tone, E. B., Phifer, J., Norrholm, S. D., Bradley, B., Ressler, K. J., Kamkwalala A., Jovanovic, T. (2011). Attention bias toward threat is associated with exaggerated fear expression and impaired extinction in PTSD. *Psychological Medicine, 42*, 533-543. doi:10.1017/s0033291711001565

Fendt, M., & Fanselow, M. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience & Biobehavioral Reviews, 23*, 743–760. https://doi.org/10.1016/s0149-7634(99)00016-0

Fendt, M., & Koch, M. (2013). Translational value of startle modulations. *Cell and Tissue Research, 354*, 287–295. https://doi.org/10.1007/s00441-013-1599-5

Golkar, A., & Öhman, A. (2012). Fear extinction in humans: Effects of acquisition–extinction delay and masked stimulus presentations. *Biological Psychology, 91*(2), 292-301. doi:10.1016/j.biopsycho.2012.07.007

Griffiths, K. M., Calear, A. L., Banfield, M., & Tam, A. (2009). Systematic Review on Internet Support Groups (ISGs) and Depression (2): What Is Known About Depression ISGs? *Journal of Medical Internet Research, 11*. https://doi.org/10.2196/jmir.1303

Griffiths, J., Ravindran, A. V., Merali, Z., & Anisman, H. (2000). Dysthymia: A review of pharmacological and behavioral factors. *Molecular Psychiatry, 5*, 242-261. doi:10.1038/sj.mp.4000697

Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology, 57*, 5–14. https://doi.org/10.1016/j.ijpsycho.2005.01.006

Harkness, K. L., & Luther, J. (2001). Clinical risk factors for the generation of life events in major depression. *Journal of Abnormal Psychology, 110*, 564–572. https://doi.org/10.1037/0021-843x.110.4.564

Hughes, M. M., Connor, T. J., & Harkin, A. (2016). Stress-Related Immune Markers in Depression: Implications for Treatment. *International Journal of Neuropsychopharmacology*. https://doi.org/10.1093/ijnp/pyw001

Joos, E. (2011). Repetitive thought in human (fear) conditioning: Strengthening the acquisition and extinction memory (Doctoral dissertation).

Kaviani, H., Gray, J., Checkley, S., Raven, P., Wilson, G., & Kumari, V. (2004). Affective modulation of the startle response in depression: influence of the severity of depression, anhedonia, and anxiety. *Journal of Affective Disorders*, *83*, 21–31. https://doi.org/10.1016/j.jad.2004.04.007

Kendall, P. C., Hollon, S. D., Beck, A. T., Hammen, C. L., & Ingram, R. E. (1987). Issues and recommendations regarding use of the Beck Depression Inventory. *Cognitive Therapy and Research*, *11*, 289–299. https://doi.org/10.1007/bf01186280

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1998). Stressful Life Events and Major Depression: Risk Period, Long-Term Contextual Threat, and Diagnostic Specificity. *The Journal of Nervous & Mental Disease*, *186*, 661–669. https://doi.org/10.1097/00005053-199811000-00001

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2020). Evidence for impaired extinction learning in humans after distal stress exposure. *Neurobiology of Learning and Memory*, *167*, 107127. https://doi.org/10.1016/j.nlm.2019.107127

Koch, M. (1999). The neurobiology of startle. *Progress in Neurobiology*, *59*, 107–128. https://doi.org/10.1016/s0301-0082(98)00098-7

Landis, C., & Hunt, W. A. (1936). Studies of the Startle Pattern: III. Facial Pattern. *The Journal of Psychology*, *2*, 215–219. https://doi.org/10.1080/00223980.1936.9917454

Lang, P. J. (1995). The emotion probe: Studies of motivation and attention. *American Psychologist*, *50*, 372–385. https://doi.org/10.1037/0003-066x.50.5.372

Lei, X., Zhong, M., Liu, Y. *et al.* Psychometric properties of the 10-item ruminative response scale in Chinese university students. *BMC Psychiatry* 17, 152 (2017). https://doi.org/10.1186/s12888-017-1318-y

Leuner, B., & Shors, T. (2013). Stress, anxiety, and dendritic spines: What are the connections? *Neuroscience*, *251*, 108–119. https://doi.org/10.1016/j.neuroscience.2012.04.021

Lissek, S., Powers, A. S., Mcclure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy*, *43*, 1391–1424. https://doi.org/10.1016/j.brat.2004.10.007

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., … Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience & Biobehavioral Reviews*, *77*, 247–285. https://doi.org/10.1016/j.neubiorev.2017.02.026

Maniam, J., Antoniadis, C., & Morris, M. J. (2014). *Early-Life Stress, HPA Axis Adaptation, and Mechanisms Contributing to Later Health Outcomes. Frontiers in Endocrinology*, *5*. doi:10.3389/fendo.2014.00073

Mazure, C. M. (1998). Life Stressors as Risk Factors in Depression. *Clinical Psychology: Science and Practice*, *5*, 291–313. https://doi.org/10.1111/j.1468-2850.1998.tb00151.x

Notarius, C. I., & Levenson, R. W. (1979). *Expressive tendencies and physiological response to stress. Journal of Personality and Social Psychology, 37, 1204–1210.* doi:10.1037/0022-3514.37.7.1204

Pavlov, I.P. 1927. Conditioned reflexes. Oxford University Press, London, United Kingdom

Rajkowska, G. (2003). Depression: What We can Learn from Postmortem Studies. *The Neuroscientist*, *9*, 273–284. https://doi.org/10.1177/1073858403252773

Rodrigues, S. M., Ledoux, J. E., & Sapolsky, R. M. (2009). The Influence of Stress Hormones on Fear Circuitry. *Annual Review of Neuroscience*, *32*, 289–313. https://doi.org/10.1146/annurev.neuro.051508.135620

Rooney, Alasdair G., et al. "Frequency, Clinical Associations, and Longitudinal Course of Major Depressive Disorder in Adults With Cerebral Glioma." *Journal of Clinical Oncology*, vol. 29, no. 32, 2011, pp. 4307–4312., doi:10.1200/jco.2011.34.8466.

Rudolph, K. D., Hammen, C., Burge, D., Lindberg, N., Herzberg, D., & Daley, S. E. (2000). Toward an interpersonal life-stress model of depression: The developmental context

ofstress generation. *Development and Psychopathology*, *12*(2), 215-234. doi:10.1017/s0954579400002066

Rudy, J. W. (2009). Context representations, context functions, and the parahippocampal-hippocampal system. *Learning & Memory*, *16*, 573–585. https://doi.org/10.1101/lm.1494409

Schuler, R. S. (1980). Definition and conceptualization of stress in organizations. *Organizational Behavior and Human Performance*, *25*, 184–215. https://doi.org/10.1016/0030-5073(80)90063

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology*, *33*, 890–895. https://doi.org/10.1016/j.psyneuen.2008.03.001

Selye, H. (1976). Stress without Distress. *Psychopathology of Human Adaptation*, 137–146. https://doi.org/10.1007/978-1-4684-2238-2_9

Shors, T., Seib, T., Levine, S., & Thompson, R. (1989). Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science*, *244*, 224–226. https://doi.org/10.1126/science.2704997

Sjouwerman, R., Niehaus, J., & Lonsdorf, T. B. (2015). Contextual Change After Fear Acquisition Affects Conditioned Responding and the Time Course of Extinction Learning—Implications for Renewal Research. *Frontiers in Behavioral Neuroscience*, *9*. doi:10.3389/fnbeh.2015.00337

Sorenson, R. D. (2007). Stress management in education: warning signs and coping mechanisms. *Management in Education*, *21*, 10–13. https://doi.org/10.1177/0892020607079985

Stam, R., Bruijnzeel, A. W., & Wiegant, V. M. (2000). Long-lasting stress sensitisation. *European Journal of Pharmacology*, *405*, 217–224. https://doi.org/10.1016/s0014-2999(00)00555-0

Steinfurth, E. C., Kanen, J. W., Raio, C. M., Clem, R. L., Huganir, R. L., & Phelps, E. A. (2014). Young and old Pavlovian fear memories can be modified with extinction

training during reconsolidation in humans. *Learning & Memory, 21*(7), 338-341. doi:10.1101/lm.033589.113

Teasdale, J. D., & Barnard, P. J. (1993). *Affect, cognition, and change: Re-modelling depressive thought*. Hove: Lawrence Erlbaum.

The American Institute of Stress. (1999). *Stress, stress, stress.* Retrieved August 2006, from https://www.stress.org/

Vrana, S. R. (1988). *Sentence imagery: attention, emotion, and the startle reflex.*

Vyas, A., Mitra, R., Rao, B. S. S., & Chattarji, S. (2002). Chronic Stress Induces Contrasting Patterns of Dendritic Remodeling in Hippocampal and Amygdaloid Neurons. *The Journal of Neuroscience, 22*, 6810–6818. https://doi.org/10.1523/jneurosci.22-15-06810.2002

Wolf, O. T. (2017). Stress and memory retrieval: mechanisms and consequences. *Current Opinion in Behavioral Sciences, 14*, 40–46. https://doi.org/10.1016/j.cobeha.2016.12.001