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Effects of cortisol on retrieval of extinction memory in individuals with social anxiety

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ABSTRACT

While exposure-based treatment for social anxiety disorder (SAD) has been shown to be effective, the high relapse rate remains a problem. Although relapse has been understood as the inability to retrieve extinction memory, the factors that influence the extent of retrieval of extinction memory have not been determined. This study aimed to examine whether the cortisol response to acute stressors in socially anxious individuals inhibits the retrieval of extinction memory, focusing on the cortisol response to acute stressors as a factor. Thirty-nine participants who scored 42 or more on the Liebowitz Social Anxiety Scale participated in the experiment for two consecutive days. On the first day, a fear conditioning task aimed at learning fear and extinction memory was administered, and on the second day, a psychosocial stress task (Trier Social Stress Test; TSST) was conducted, followed by an extinction retrieval test. The results indicated that cortisol responsiveness (Responder/ Non-responder) was not associated with the retrieval of extinction memory indexed by subjective and physiological measures. However, a supplementary analysis revealed that the total amount of cortisol secretion was associated with attenuated retrieval of extinction memory. These findings suggest that the total cortisol secretions, rather than cortisol responsiveness to the acute stressor, may play a role in relapse.

1. Introduction

Social anxiety disorder (SAD) is characterized by a fear of negative evaluations by others or being scrutinized during social interactions [1]. Exposure-based therapies are effective treatment approaches for SAD [10]. However, relapse of anxiety responses after successful therapies remains a major limitation to current therapies. For instance, in some cases, a relapse of anxiety responses rate of up to 62% has been documented [7].

Extinction learning comprises the foundation of exposure-based therapies. Pavlovian fear conditioning paradigm is valuable for investigating fear and extinction learning [47]. In this paradigm, during acquisition training, a neutral stimulus (conditioned stimulus; CS) is repeatedly paired with an aversive stimulus (unconditioned stimulus; US). This usually results in the formation of the original CS-US association (fear memory). Subsequently, during extinction training, the CS is repeatedly presented without the US, which usually results in the

formation of the inhibitory CS-noUS association (extinction memory). During the extinction retrieval test, following acquisition and extinction training, the anxiety responses (conditioned responses; CR) for CS that is measured depends on which of the two opposing and co-existing memory traces (fear memory vs. extinction memory) is dominant. Thus, it is referred to as retrieval deficit of extinction memory when the CR is strong and dominance of the fear memory trace is assumed. Based on this understanding of relapse, several approaches, for instance, multiple contexts and retrieval cues, have been utilized to enhance the retrieval of extinction memory after therapies in pre-clinical and clinical samples (reviewed in [8]). However, these approaches during exposure-based therapies have not been as effective as the theory suggests (e.g., [12]). Therefore, to prevent relapse of anxiety responses, it may be useful to examine factors that underpin individual differences in the retrieval of extinction memory.

In past studies, acute stress has been reported to impair the recall of extinction memory [39]. Under acute stress, the activation of the

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hypothalamus-pituitary-adrenal (HPA) axis leads to the release of cortisol [19]. Cortisol is a potent modulator of learning and memory [43] and interferes with retrieval of memory in particular [48]. For instance, a dose of cortisol impairs the retrieval of extinction memory [21]. Recent review articles have suggested that acute stressor or cortisol presentation prior to retrieval test may interfere with the retrieval of extinction memories [32]. Considering these studies, stress-induced cortisol before extinction retrieval test may impair retrieval of extinction memory and promote relapse of the anxiety response. However, previous studies have been dominated by the presentation of acute stressors or exogenous cortisol administration, and no studies have examined relapse with a focus on stress-induced cortisol reactivity.

The HPA axis is known to be highly responsive to stressors of an uncontrollable and social-evaluative nature [11]. Since one of the core features of social anxiety is fear of negative evaluations by others, a recent review revealed that individuals with SAD show an increased cortisol response to psychosocial stress [14].

Therefore, cortisol induced by social-evaluative stressor may impair the retrieval of extinction memory and promote relapse of anxiety response in individuals with subclinical social anxiety. However, no studies have examined relapse of anxiety responses from the perspective of the effect of stress-induced cortisol on retrieval of extinction memory in social anxiety. Thus, we hypothesized that poorer retrieval of extinction memory would be observed during extinction retrieval test among those with greater cortisol responses to a social-evaluative stressor.

2. Methods

2.1. Participants

An a priori power analysis was performed using G*power 3.1 [15] to calculate a sample size sufficient to test the first hypothesis (i.e., the effect of cortisol on retrieval of extinction memory). We aimed to detect a medium-sized effect of cortisol on retrieval of memory as reported in a meta-analysis by [18]. The power analysis yielded a total of 30 participants to achieve a power of $1-\beta \geq 0.90$ to detect a significant interaction comprising a two-way mixed ANOVA.

Prior to participating in the experiment, applicants completed the Japanese translated self-report version of the Liebowitz Social Anxiety Scale (LSAS; [2,16]). Individuals who met any of the following criteria were considered ineligible: (a) a score below 42 on the LSAS, indicating low levels of social anxiety symptoms; (b) a history of a diagnosed psychiatric disorder; (c) stressful experiences just prior to the experiment; (d) a history of smoking; (e) use of medications that could affect cortisol responses (e.g., oral contraceptives, β-blockers); (f) suffering from severe sleep disturbance or fatigue; and (g) irregular menstruation or out of the luteal phase (for women). All the female participants were tested during the luteal phase of their menstrual cycle to minimize the impact of menstrual cycle variation in stress hormones [33]. Therefore, we assumed that the effects of gender differences noted in previous studies would be minimal. Forty-two healthy participants were recruited through advertisements posted around the university campus. Of these, three participants were excluded from all the analyses due to termination of the experiment because they did not show up for the second testing session ($n = 1$) and withdrawal due to a negative reaction from the tasks ($n = 2$). Thus, the final sample consisted of 39 individuals (female: 25, male: 14; age: 19–29 years; mean age $\pm$ sd: 21.6 $\pm$ 2.1 years) who met the criteria described above and completed all the tests. Participants were asked to abstain from vigorous exercise, alcohol, caffeine, and food for 1 h prior to study participation. Written informed consent was obtained from all participants, and they were informed that they could withdraw from the study at any time. Participants were provided with a book coupon worth 2500 Japanese yen to participate in the study. The study was approved by a local ethics committee and conducted in accordance with the Declaration of Helsinki.

2.2. Measures

2.2.1. Self-report measures

Participants' demographic information was collected during the adaptation phase of starting the experiment. More detailed levels of social anxiety symptoms were assessed using the Social Phobia Scale (SPS; [31]). The SPS is a scale that assesses anxiety about performance in public and social interaction. It consists of 20 items rated on a five-point Likert scale (range: 0–80). Levels of depressive symptoms were assessed using the Center for Epidemiological Studies Depression Scale (CES-D; [38]). It consists of 20 items assessed on a four-point Likert scale (range: 0–60). Furthermore, the level of core features of social anxiety was assessed using the Short Fear of Negative Evaluation Scale (SFNE; [41]). SFNE is a scale that assesses fear of negative evaluation by others. It consists of 12 items rated on a five-point Likert scale (range: 12–60). We used the Japanese translated and validated versions of the SPS [20] and CES-D [45]. In addition to these self-report measures, the visual analog scale (VAS) was used to measure subjective state anxiety during the experiment. Anchor values of 0 and 100 were defined as "not at all" and "extremely" anxious, respectively.

2.2.2. Fear conditioning and extinction procedures

A modified version of the differential fear conditioning paradigm, as described in [24] was applied, consisting of stimulus habituation, fear acquisition, and subsequent fear extinction training (Fig. 1). For this task, two neutral and fearful facial expressions each, one of each male and one of each female, were taken from the ATR facial expression database [3]. Neutral faces (female: F10-NE-1, F13-NE-1; male: M1-NE-1, M5-NE-1) served as the conditioned stimuli (CS), while fearful faces (female: F10-FE-1, F13-FE-1; male: M1-FE-1, M5-FE-4) paired with two screams (female: #276, 277; male: #275, 292) taken from the International Affective Digitized Sounds (IADS; [5]) served as the unconditioned stimuli (US). Because participants with social anxiety were more likely to have anxiety about the opposite sex, we used stimuli of the opposite sex of the participants' gender [6]. One neutral face (CS+) was coupled with the UC and the other was never paired (CS−) for each gender of the models. Allocation of CS+ and CS− was counterbalanced and presented the opposite gender to the participants. Participants were instructed to pay attention to the screen and imagine that they had met the person presented on the screen. During the habituation phase, both stimuli (CS+, CS−: 8 s) were presented in eight trials, four trials each. During the fear acquisition phase immediately after the habituation phase, CS+ was followed by US starting 8 s after CS + onset (CS+ and US: 15 s, 95 db) in 12 trials, whereas CS− was never paired with the US in 12 trials (CS− and noUS: 15 s). During the fear extinction phase immediately after the acquisition phase, both stimuli (CS+, CS−: 8 s) were presented in 24 trials, 12 trials each. A black screen with a white fixation cross (20 mm × 20 mm) was shown during the 1 s gap between the end and beginning of the CS presentation.

2.2.3. Extinction retrieval test

On day 2, we used a standard acute psychosocial stress test, namely the Trier Social Stress Test (TSST), which required participants to deliver a speech and perform mental arithmetic in front of two audiences [22]. Twenty minutes after TSST, when cortisol levels began to rise, participants were set up for an extinction retrieval test. During the extinction retrieval test, no further instructions were given. Participants were presented with both stimuli (CS+, CS−: 8 s) in 24 trials, 12 trials each.

2.2.4. Subjective ratings

Immediately after the habituation phase, participants rated the percentage of anxiety ("When you see these faces, how strong is your anxiety?"; 0 = no anxiety; 100 = very severe anxiety), valence ("How positive/negative are these faces to you?"; 0 = very positive; 100 = very
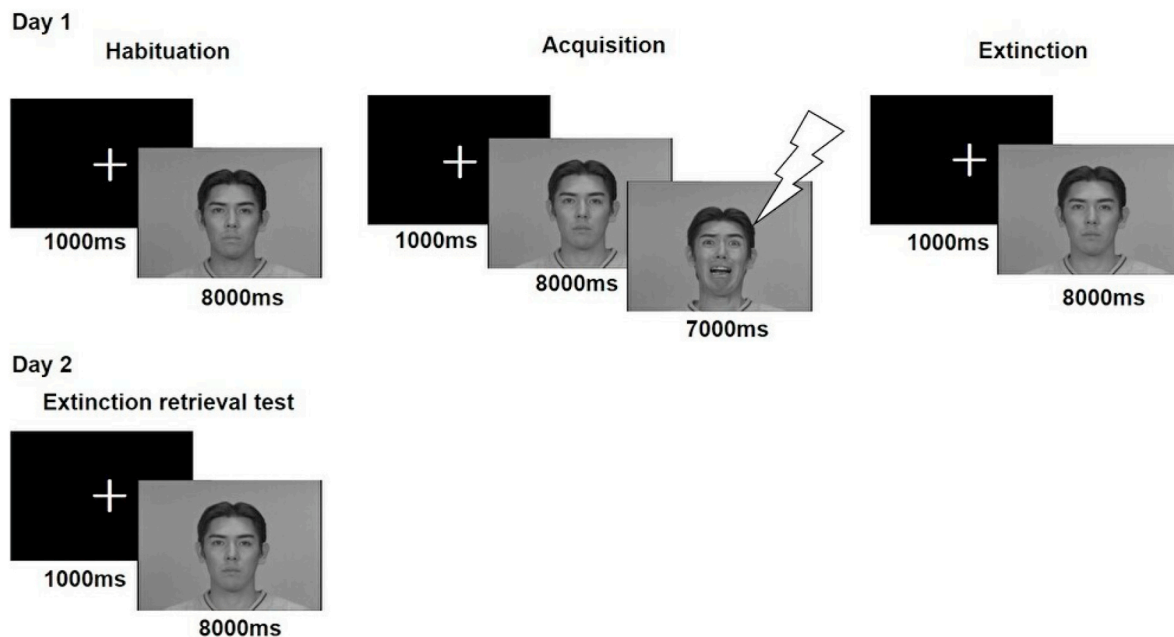
Fig. 1. Fear conditioning paradigm and extinction retrieval test.

negative), arousal ("How arousing are these faces to you?"; 0 = no arousal; 100 = very arousal) on a visual analog scale (VAS). Additionally, after the fear acquisition, fear extinction phases, and extinction retrieval test, participants rated the percentage of US expectancy ("Do you expect a scream to follow these facial expressions?"; 0 = definitely not; 100 = definitely).

2.2.5. Skin conductance response (SCR)

SCR was recorded at 1000 Hz with a commercial SCR coupler and amplifying system using Ag/AgCl electrodes filled with the same isotonic electrolyte medium as for the electrical stimulation. Electrodes were placed on the index and middle finger of the second joint of the left hand.

2.2.6. Pupil diameter

Pupil diameter was recorded with the Gazepoint GP3 system at a corresponding binary quality factor (valid/invalid) at 150 samples/s.

2.2.7. Cortisol levels

Participants were asked to draw saliva from their mouths for 2 min and drool into a specimen tube through a 4 cm long straw (passive drool). Saliva samples were frozen in a freezer at temperatures below $-20\,^{\circ}C$ until assay. Salivary cortisol levels were measured using enzyme-linked immunoassay using a commercial kit from Salimetrics (State College, PA, USA). The inter-assay coefficient of variation across all assays was 6.5%, and the intra-assay coefficient of variation was 4.4%.

2.3. Procedure

On the day before the experiment, to control for confounding factors affecting cortisol levels, participants were instructed to (a) abstain from caffeine on the day of the experiment, and (b) limit eating, drinking, brushing teeth, and strenuous exercise immediately before the experiment. To control for circadian variation in cortisol activity, all experiments were performed in the afternoon (between 1200 h and 1930 h).

On day 1, at the beginning of the experiment, participants provided informed consent and then completed a demographic questionnaire in approximately 10 min. Subsequently, participants sat in a quiet room for 10 min [46]. Participants then underwent a fear conditioning task for approximately 15 min. On day 2, participants sat in a quiet room for 10

min, like day 1. Next, the participants received instructions for the TSST. They had 10 min to prepare their speeches, and the TSST included a 5 min speech and a 5 min mental arithmetic task. After the TSST, participants had 10 min to rest. Twenty minutes after the TSST, participants underwent the extinction retrieval test. During the experiment, participants were not allowed to eat or drink anything but a little water. Saliva collection and assessment of state anxiety were conducted at six time points: before fear conditioning task (On day 1; T1), after fear conditioning task (On day 1; T2), baseline (On day 2; T3), after speech preparation (On day 2; T4), after the TSST (On day 2; T5), and after extinction retrieval test (On day 2; T6). All experimental procedures are illustrated in Fig. 2.

2.4. Statistical analyses

For the TSST, a one-way repeated measures ANOVA with time was conducted for subjective state anxiety to confirm that it successfully served as a social-evaluative stressor. If the assumption of sphericity was not met in the repeated measures analysis, a Greenhouse-Geisser correction for non-sphericity was applied. For cortisol levels, we calculated the cortisol response rate using the criteria proposed by [35] where those who exhibited a cortisol increase of 15.5% or more from baseline were considered Responders and those who did not exhibit a cortisol increase were considered Non-responders.

For SCR and pupil diameter, we quantified them within each experimental phase (habituation, acquisition, extinction, and extinction retrieval test). In addition, trial-by-trial analyses were conducted during the acquisition and extinction phases. For the extinction retrieval test, we calculated the mean of the first two trials [17], to not reflect the "re-extinction" effect, which could occur later in the extinction retrieval test. For SCR, 36 people were included in the analysis, excluding three who were unable to acquire data properly due to problems in equipment. The maximum value minus the minimum value was calculated within 1–8 s after the stimulus was presented [23]. For outlier analysis, SCR was z-standardized and defined for each participant separately over all data (Z > 3.00). Outliers and missing data due to technical difficulties were replaced by a linear trend. Each participant's SCR was pre-processed using MATLAB (version 2019, MathWorks, Natick, USA) prior to analysis by low-pass filtering (cutoff frequency 25 Hz) and mean -value smoothing using a 3-sample window [39].
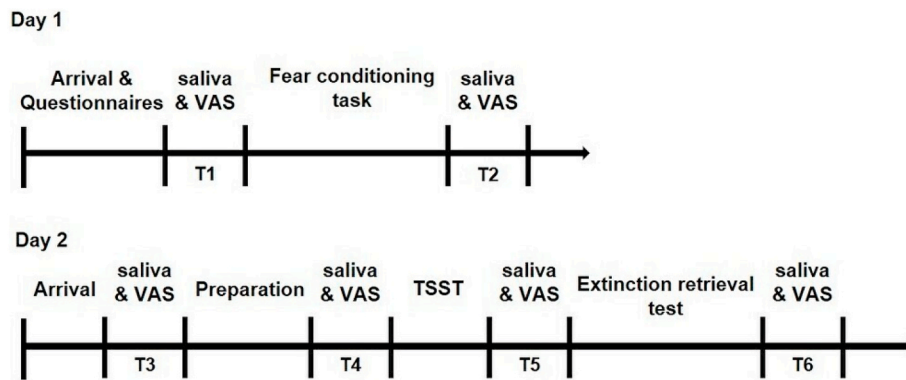
Day 1

Fig. 2. Overview of the testing timeline.

For pupil diameter, 37 participants were included for analysis, excluding two who were unable to acquire data properly due to problems in equipment. Pupil diameter was calculated by subtracting the mean pupil diameter for the 0.5 s before CS onset from the maximum pupil dilation just before US stimulus onset for the acquisition phase [25]. For habituation, extinction, and extinction retrieval test phases, pupil diameter data were calculated by subtracting the mean pupil diameter for the 0.5 s before CS onset from the maximum pupil dilation in the 1 s before CS offset [26]. We used the one with the lowest number of invalid in the left and right eye. To control for variability across subjects, the entire pupil data per participant were z-transformed. Each participant's pupil diameter was preprocessed using MATLAB prior to analysis and smoothed with a 200 ms sliding window [25].

Successful fear conditioning was defined as there being a statistically significant difference in response to the CS + compared to the CS− at the acquisition phase. Successful extinction was defined as no significant difference in response to the CS + compared to the CS− at the extinction phase [40], and there was a significant decrease in the response to CS+ from the acquisition to the extinction phase. Physiological indices (SCR and pupil diameter) were measured during each phase (habituation, acquisition, extinction, and extinction retrieval test) and the average of each phase was calculated. Subjective ratings (anxiety, valence, arousal, US expectancy) were measured after the end of each phase.

Further, to supplement the ANOVA results with cortisol responsiveness as a dichotomous variable, we conducted a correlation analysis between cortisol secretion (area under the curve concerning ground [AUCg] and area under the curve concerning increase [AUCi]) and each index.

As definitions of AUCg and AUCi, total cortisol secretion (AUCg) included amounts other than those in response to acute stress. It was calculated using three samples (T3, T4, and T5) before the extinction retrieval test on day 2. Specifically, it was calculated using the following formula, and 10 and 20 reflect the time between each sample:

Total cortisol secretion (AUCg) = ((T3+T4)*10+(T4+T5)*20)/2

Next, cortisol reactivity secretions to the stress (AUCi) focused on the amount of secretion in response to acute stress. It was calculated using the baseline secretion from the total cortisol secretion before the extinction retrieval test on day 2. Specifically, it was calculated using the following formula, and 30 reflects the time between T3 and T5:

Cortisol reactivity secretions to the stress (AUCi) = AUCg – (T3*30)

We calculated the correlation between total cortisol secretions (AUCg), cortisol reactivity secretions to the stressor (AUCi), differential values of CS+ and CS– in subjective indices (extinction phase-extinction retrieval test), and differential values of CS+ and CS– in physiological indices (last trial of the extinction phase – average of the first two trials of the extinction retrieval test).

All analyses were conducted in R 3.6.0. The significance levels were set at 0.05 (two-tailed).

3. Results

3.1. Preliminary analysis

The overall cortisol response rate to the TSST (showing increase) was 64.1%, which was almost comparable to the response rates in previous studies (>70.0%; [22]). Descriptive statistics for demographic questionnaires for Responders and Non-responders are summarized in Table 1. There were no significant differences in age, gender ratio, body mass index (BMI), and demographic questionnaire scores.

3.2. Manipulation check

For subjective state anxiety, one-way repeated measures ANOVA revealed a significant effect of time (F (3, 114) = 29.74, ε = 0.44, $p < $ .001). Contrast analysis using Holm's correction for multiple comparisons revealed that participants exhibited elevated anxiety in anticipation of the TSST (at T4; $p < $ .001), which lasted even after they had completed the TSST (at T5; $p = $ .003). Additionally, for cortisol responses, a two-way 2 (responder type: Responder, Non-responder) × 4 (time: T3, T4, T5, T6) mixed design ANOVA on cortisol levels revealed a significant interaction (F (3, 111) = 16.08, $p < $ .001). Post-hoc analyses using Holm's correction revealed that the cortisol levels were higher at T5 and T6 (after TSST) than at T3 and T4 (before TSST; $p < $ .01 for all) among Responders. These results indicated that cortisol increased significantly only among Responders.

For fear acquisition, all subjective ratings (anxiety, valence, arousal, US expectancy) as well as pupil diameter were higher for CS + than for CS− at the acquisition phase (all $ts < 12.53$, $ps < .001$). However, SCR did not show a significant difference between CS+ and CS− ($t = 0.64$, $p = .53$). Analysis of trial-by-trial ratings for this phase with trial order as within-subjects factor revealed significantly higher pupil diameter across trials for CS+ (F (23,828) = 4.45, $p < $ .001), and no significant

Table 1

Group means (±SD) for demographics and questionnaires scores.

	Responder($n = 25$)	Non-responder($n = 14$)	t/χ^2	p
sex(M: F)	11 : 14	3 : 11	1.99	.16
age	22.0(2.22)	20.9(1.66)	1.68	.10
BMI	20.52(2.73)	20.64(2.34)	0.14	.89
SPS	32.32(12.29)	30.36(14.50)	0.45	.66
CES-D	18.72(8.59)	17.07(9.61)	0.55	.59
LSAS	70.48(19.06)	71.86(18.71)	0.22	.83
SFNE	45.44(5.87)	48.86(5.38)	1.80	.08

Note. SPS = Social Phobia Scale; CES-D = Center for Epidemiologic Studies Depression scale; LSAS = Liebowitz Social Anxiety Scale; SFNE = Short Fear of Negative Evaluation Scale.

difference between CS+ and CS− for SCR ($F (23,805) = 0.66$, $p = .88$).

For fear extinction, all subjective ratings were higher for CS + than for CS− (all $ts < 5.86$, $ps < .001$). As expected, the difference between CS+ and CS− in the extinction phase (anxiety: $d = 0.69$; valence: $d = 0.83$; arousal: $d = 0.62$; US expectancy: $d = 0.59$) tended to be smaller than in the acquisition phase (anxiety: $d = 1.71$; valence: $d = 2.06$; arousal: $d = 2.72$; US expectancy: $d = 2.33$). Furthermore, the response to CS+ was reduced from the acquisition to the extinction phase for all subjective ratings (all $ts < 9.15$, $ps < .001$). For physiological indices of fear, there were no significant differences between CS+ and CS− at the extinction phase (all $ts < 1.04$, $ps > .31$). Analysis of trial-by-trial ratings for this phase with trial order as within-subjects factor revealed no significant difference between CS+ and CS− (pupil diameter: $F (23,828) = 1.15$, $p = .32$; SCR: $F (23,805) = 0.71$, $p = .82$). Furthermore, for pupil diameter, the response to CS+ was reduced from the acquisition to the extinction phase ($t = 3.14$, $p = .003$).

To summarize, the subjective ratings and pupil diameter supported the successful fear conditioning and extinction, but the SCR did not. However, because pupil diameter has higher retrodictive validity than SCR [37], we assumed that fear acquisition and extinction were successful. *Means* and standard deviation (*SDs*) are summarized in Table 2 and each trial in each phase is presented in Supplemental file 1.

3.3. Extinction retrieval test

For subjective ratings, repeated measures ANOVA of stimulus type (CS+, CS−) and between-subject factors of responder type (Responder, Non-responder) revealed significant main effects of stimulus type (all $Fs (1,37) < 49.49$; $ps < .001$). However, we did not observe a responder type × stimulus type interaction (all $Fs (1,37) < 0.87$, $ps > .36$; Fig. 3).

For SCR and pupil diameter, repeated measures ANOVA of type and between-subject factors of condition revealed no main effects of stimulus type, responder type, and interaction (SCR: all $Fs (1,34) < 1.92$, $ps > .18$; pupil diameter: all $Fs (1,35) < 2.80$, $ps > .10$; Fig. 3).

3.4. Supplemental analyses

To assess whether cortisol was related to retrieval of extinction memory supplementally, we conducted a correlation analysis between total cortisol secretions (AUCg), cortisol reactivity secretions to the

Table 2
Each index score ($\pm SD$) during the fear conditioning paradigm.

	Subjective ratings	SCR	Pupil diameter
Habituation			
CS+	Anxiety 33.85(21.63)	1.34(0.31)	0.08(0.12)
	Valence 50.26(11.99)		
	Arousal 27.10(19.63)		
CS−	Anxiety 34.67(21.31)	1.30(0.34)	0.05(0.14)
	Valence 51.67(8.86)		
	Arousal 26.69(21.83)		
Acquisition			
CS+	Anxiety 77.46(13.56)	1.32(0.21)	0.26(0.22)
	Valence 83.28(11.64)		
	Arousal 86.05(13.44)		
	US expectancy 89.82(13.07)		
CS−	Anxiety 43.05(25.10)	1.33(0.19)	0.08(0.14)
	Valence 50.92(19.02)		
	Arousal 32.33(24.64)		
	US expectancy 40.59(26.93)		
Extinction			
CS+	Anxiety 61.87(22.31)	1.30(0.21)	0.19(0.19)
	Valence 64.82(17.70)		
	Arousal 45.33(25.28)		
	US expectancy 58.59(26.64)		
CS−	Anxiety 45.36(25.35)	1.27(0.17)	0.19(0.20)
	Valence 50.36(17.44)		
	Arousal 30.03(24.35)		
	US expectancy 43.05(26.27)		

stressor (AUCi) on day 2, and differential values of CS+ and CS− between the extinction phase to extinction retrieval test phase for all indices (see Table 3).

For subjective ratings, the Spearman correlation between AUCg and differential values of CS+ and CS− (subjective anxiety and US expectancy) was positive (anxiety: $rho = 0.37$, $p < .05$; US expectancy: $rho = 0.33$, $p < .05$). For AUCi, no significant correlation was identified for any of the indices (anxiety: $rho = -0.02$, $p = .90$; valence: $rho = -0.04$, $p = .83$; arousal: $rho = -0.03$, $p = .87$; US expectancy: $rho = 0.04$, $p = 80$).

For pupil diameter and SCR, the Spearman correlation between cortisol secretions (AUCg and AUCi) and differential values of CS+ and CS− for all indices did not reach significance (SCR: AUCg: $rho = 0.00$, $p = .99$, AUCi: $rho = -0.06$, $p = .74$; pupil diameter: AUCg: $rho = -0.25$, $p = .14$, AUCi: $rho = -0.14$, $p = .40$). Each scatter plot is described in Supplemental file 2.

4. Discussion

This study aimed to examine whether cortisol following a social-evaluative stressor inhibited retrieval of extinction memory in individuals with subclinical social anxiety. We hypothesized that poorer retrieval of extinction memory would be observed in those with greater cortisol responses to a social-evaluative stressor. However, the results showed that the extent of retrieval of extinction memory did not differ in either responder type for any of the indices. Thus, the hypothesis that an acute cortisol response promotes relapse of anxiety responses through inhibition of retrieval of extinction memory was not supported. The results of the supplemental correlation analysis with AUCi (cortisol secretion in response to acute stress) also supported this view.

Our results concerning the effects of cortisol response to acute stress on the retrieval of extinction memory contradict clinical reports that state acute stress or cortisol impairs the retrieval of extinction memory [21,39]. However, this discrepancy can be resolved by considering that the extent of extinction learning was insufficient. In this study, successful fear extinction was defined as no significant difference in response magnitude to the CS + compared to the CS− at the end of the extinction phase [40]. As a result, there was a significant decrease in the response to CS+ from the acquisition to the extinction phase. However, on the subjective ratings, anxiety responses to CS+ during the extinction phase was significantly higher than that of CS−. Similar results have been reported in studies with participants with a social anxiety disorder [24]. Therefore, it is insufficient as to the extent of extinction learning, and the effect of cortisol response on extinction memory may not have been clearly demonstrated. For instance, fear-relevant CS, such as facial expression, has been suggested to be a resistance to extinction learning as compared to fear-irrelevant CS, such as shapes [28,36]. However, extended extinction training, which utilized a larger number of extinction trials, can be effective at reducing stimulus valence ratings [27,29]. [27] used 32 trials. Future studies that use such a method may be useful for more successful extinction learning.

Although the hypothesis was not supported, the association between total cortisol secretions (AUCg) and differential values of CS+ and CS− (subjective anxiety and US expectancy) was positive. This result suggests that not only the response to the acute stressor (difference between individual cortisol samples) but also the baseline secretion of cortisol (overall distance of cortisol samples from the ground) may have enhanced the inhibition of retrieval of memory. For example, in [42], there was an inverted U-shaped dose-response relationship between the amount of cortisol administration and memory recall. [42] suggest that moderate cortisol administration enhanced memory recall, whereas low or high doses inhibited memory recall. Although there is a difference between administration and secretion, this finding of [42] may suggest the usefulness of focusing on the total amount of secretion when considering relapse of anxiety. One potential source of high total cortisol secretion is a dysfunction of the HPA system. For instance, chronic stress may lead to a breakdown in the negative feedback system of cortisol
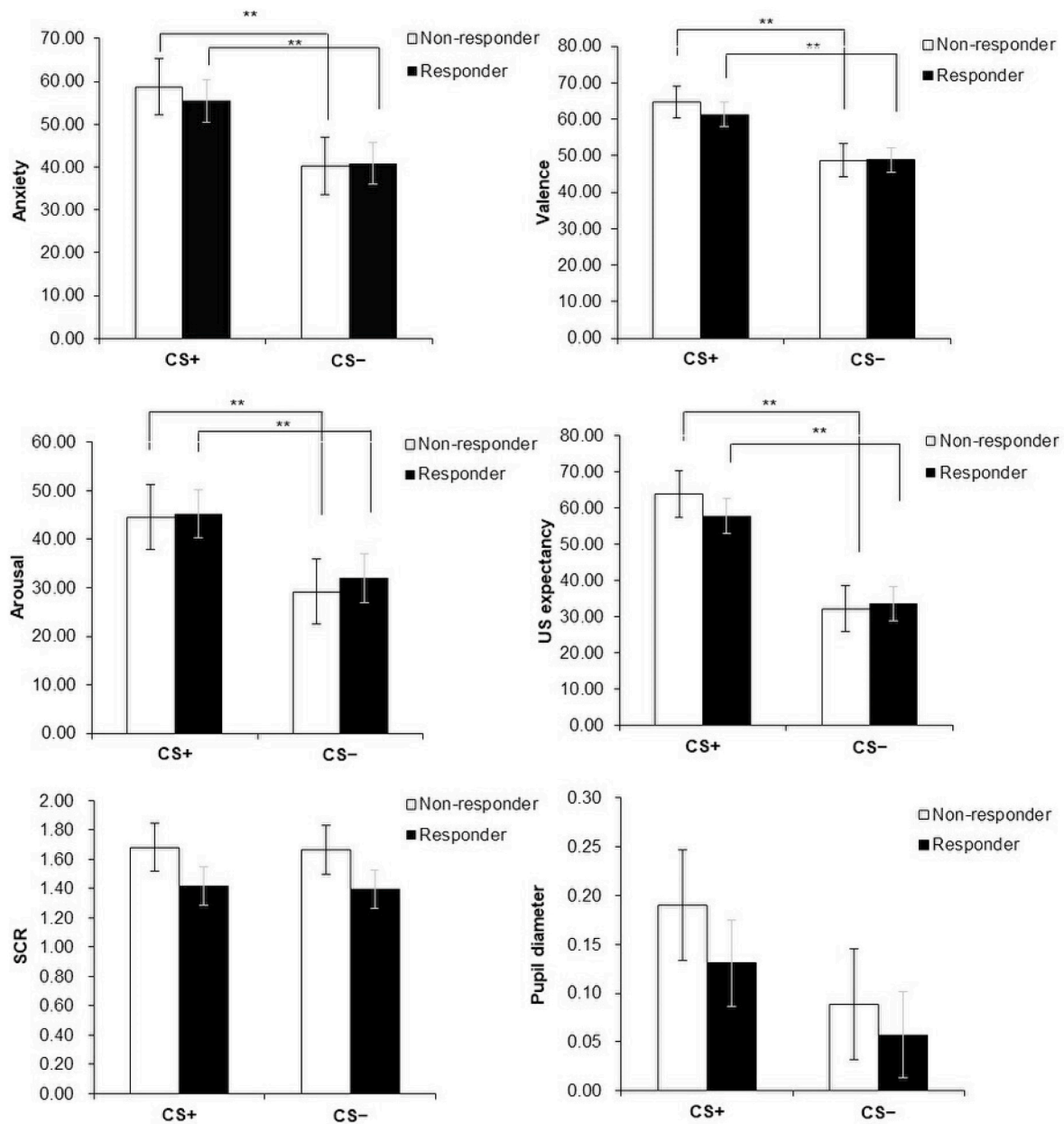
Fig. 3. Subjective and physiological indices ratings during extinction retrieval test.
Note. **$p < .001$.

Table 3
Spearman correlation between cortisol secretions (AUCg and AUCi) and the differential values of CS+ and CS− for all indices.

	AUCg		AUCi
Subjective ratings			
Anxiety	.37	*	−.02
Valence	.25		−.04
Arousal	.23		−.03
US expectancy	.33	*	.04
SCR	.00		−.06
Pupil diameter	−.25		−.14

Note. *$p < .05$.

secretion, resulting in higher secretion end-of-day [34]. Thus, chronic stress may be a factor in the background of high total cortisol secretion during an acute stress situation. In considering relapse, it may be useful

to consider the total cortisol secretion including baseline, rather than just the response to the acute stressor.

Our study has several limitations. First, since a control group was not used in this study, we cannot disregard the possibility that other factors may have influenced cortisol reactivity. The present study examined the effect of differences in cortisol responsiveness by stressor on relapse, which has not been examined before. Because Responders and Non-responders were nearly homogeneous except for reactivity, the present study was able to examine the effect of differences in cortisol responsiveness to acute stressors on retrieval of extinction memory. However, to more closely examine the effect of cortisol responsiveness to acute stressors on relapse, setting a non-stress control group would be valuable. Second, the results of this study were derived from a non-clinical university student sample, and the generalizability of the results to clinical populations may be limited. Replication of this study with an actual clinical sample is desirable. Third, during the fear conditioning paradigm and extinction retrieval test, SCR and pupil diameter were

measured during each phase, while the subjective ratings asked for answers after the end of each phase, suggesting that retrospective bias may have occurred. It has been reported that retrospective assessment is subject to multiple systematic distortions (i.e., affective valence effect, mood-congruent memory effect, duration neglect, peak-end rule) as it is based on storage and recollection of memories of the original experience or the behavior that are of interest [13]. Thus, in the future, it will be possible to increase the validity of the answers by using a method that allows immediate answers, such as the event-sampling approach. Fourth, in this study, successful fear conditioning and extinction were not supported by SCR. The characteristics of SCR may explain it. SCR has been shown to reflect mainly the degree of arousal and rapid habituation [4,9]. Indeed, the discrimination validity of SCR between CS+ and CS− has recently been rated as moderate [37], which gives further support for this view. Fifth, in this study, a 10-min rest period was provided to eliminate the effect of potential cortisol confounders, but this may have been insufficient. Therefore, a longer rest period may be necessary in future studies. Finally, it is possible that the sample size was not sufficient for comparing responders to non-responders. Such a comparison has been considered an important analysis for clarifying the role of cortisol in the context of stressor exposure [44]. However, the cortisol response rate observed in the present study (64.1%) resulted in an imbalanced allocation of participants for two groups and a relatively small number of non-responders. Although there have been some observations with a cortisol response rate of almost 50% (e.g. [30]), we would normally expect a greater number of participants for the responders group. In future studies, it will be desirable to design a sample size determination in consideration of the cortisol response rate.

Notwithstanding these limitations, this study suggests the cortisol may inhibit retrieval of extinction memory and facilitate relapse of anxiety responses in individuals with subclinical social anxiety. Future studies may be necessary to examine the factors that influence the relationship between stress-induced cortisol response and retrieval of extinction memory. Such a study could lead to fundamental findings to prevent relapse in social anxiety.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100060.

Declaration of competing interest

None.

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