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Social evaluation under stress: Does acute stress affect social attributions and eye gaze?

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ABSTRACT

Acute stress has been found to elicit pro-social, anti-social or null responses in humans. The causes for these contradicting findings are currently poorly understood, and may rise from subjects' characteristics, such as sex or hormonal status, as well as stimuli-based traits, such as group membership. In the current study, 120 subjects performed either the Trier Social Stress Test or a control (non-stress inducing) condition, followed by ranking displayed faces according to several attributes (e.g., trustworthiness, attractiveness, dominance). Participants' eye gaze was also tracked while viewing facial stimuli. We examined how acute stress interacts with participants' sex, female participants' hormonal status (hormonal contraceptives, early-follicular phase and mid-luteal phase), and the observed faces' social group (ethnicity-based in-group or out-groups). In general, frequentist and Bayesian analyses showed that acute stress exposure did not affect social attributions or gaze behavior, nor did it interact with subjects' sex or the group membership of the observed faces. Interestingly, sub-group analyses showed that in females, acute stress interacted with hormonal status to yield heterogenous anti-social effects, such as post-stress reductions in perceived trustworthiness in the early-follicular phase, and lower perceived attractiveness in the mid-luteal phase. Given the small sample sizes for the sub-groups, these results should be viewed as preliminary until further replicated. Our results highlight the necessity for large-scale studies, particularly in females, to further refine existing theories regarding the nature and contexts by which acute stress elicits changes in social cognition and behavior.

1. Introduction

Acute stress gives rise to a wide array of human responses, with pervasive effects on behavior, affect, and cognition [1]. While psychological and physical stressors have different triggers [2], it is well-established that psychological threats, similarly to physiological threats, can initiate a neuroendocrine and behavioral response aimed at diverting resources to ensure self-protection [3]. There has been growing interest over the last decade in regard to the effects of acute stress on social cognition and behavior. To date, the nature of these effects remains inconclusive (see Ref. [4] for an extensive review).

Leading theories in evolutionary psychology offer two apparently conflicting perspectives as to how individuals react to others under stress. The Fight-or-Flight theory [5], more-recently updated to Freeze-Fight-Flight [6], suggests individuals overcome threats through a highly effective biobehavioral response. Within milliseconds of the appearance of a perceived threat, the sympathetic nervous system primes the body for action via the release of adrenaline and noradrenaline, resulting in wide-ranging effects to multiple organ systems (e.g. increased heart rate, pupil dilation, constriction of gastrointestinal tract, and diversion of blood to skeletal muscles). If the stressor is deemed significant or threatening enough, the Hypothalamic-Pituitary-Adrenal axis initiates a hormonal cascade, resulting in the secretion of the end product cortisol. These processes support the behavioral responses of freezing, fleeing or fighting – physically and/or psychologically. The complimentary Tend-and-Befriend model [8], proposes a female-specific stress response mechanism, whereas females produce the necessary social safety net required to ensure their own and their

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children's survival by affiliative behaviors of bonding with and approaching others.

While some studies have found evidence for stress-induced tend-and-befriend prosocial responses [9], others have observed fight-or-flight anti-social responses [10]; and still others found that social responses remain unaffected by stress exposure [11]. Two key factors may account for the conflicting findings. First, most studies examining social responses to stress have been conducted on males, however, it may be that social responses to stress are sex-specific [8]. Moreover, both social functioning and stress reactivity are affected by females' menstrual cycle and use of hormonal contraceptives. For example, females in the early-follicular phase (days 1–8 following menstruation onset), when levels of estrogen and progesterone are relatively low, are more willing to cooperate than females in the mid-luteal phase (days 18–24), when estrogen and progesterone levels are higher [12]. Hormonal contraceptive use, which keeps levels of progesterone and estrogen steady throughout the month, also alters social functions, but more research is needed to determine these effects [13]. While females using hormonal contraceptives tend to show blunted cortisol reactivity in response to stress compared to naturally cycling females [14], findings regarding how menstrual cycle phase regulates cortisol reactivity are inconsistent. Some studies have observed that stressed females in the follicular phase showed a more attenuated cortisol response compared to females in the luteal phase [15], while others showed a stress-induced cortisol increase in the follicular phase, but not in the luteal phase [16].

The second factor is the target of the social response. Although humans tend to categorize others into distinguished groups from a very early stage of development [17], overwhelmingly, existing stress research is conducted under laboratory conditions where the target of the interaction is anonymous, leaving the intergroup context poorly explored. While interacting with stigmatized-others may trigger a sense of threat and anxiety [18], there is little evidence on how *incidental* stress affects social interactions towards out-group vs. in-group members. In one of the few studies to examine this question, acute stress facilitated anti-social attributions such as reduced trustworthiness toward out-group members in a male-only sample [19]. Consistent with this, elevation in cortisol levels has been found to increase activation in brain limbic regions associated with threat-monitoring [20], coupled with a shift from deliberative to intuitive thinking [21]. Higher cortisol levels can therefore make people more visceral and easily threatened, which may bare negative consequences when encountering an out-group member.

The current study aimed to test these factors systematically. We assessed socio-cognitive responses to psychosocial acute stress in equal groups of males and females. To account for fluctuating hormones levels, we tested females in three pre-defined hormonal statuses: free cycling females in the early-follicular phase, free cycling females in the mid-luteal phase, and females using hormonal contraceptives. Both before and after stress exposure, participants – Mizrahi and/or Ashkenazi Jews – rated faces of individuals from one of three ethnic groups: Mizrahi and/or Ashkenazi Jews, Ethiopian Jews, and Palestinian Israeli citizens. Participants rated both female and male targets for trustworthiness, attractiveness, dominance, and industriousness. This social attribution task allowed us to examine social responses without the potential confound of monetary reward, and to use salient natural groups when testing intergroup bias effects.

To complement the explicit assessments of trait attributes with a more autonomous socio-cognitive measure, participants' eye gaze was captured using an eye-tracking device. This measure could be considered a non-volitional response, as participants do not have full conscious access to their gaze patterns [22]. Direct fixations of gaze to the eye region have been linked to higher socio-cognitive function [23], and are observed following exogenous administration of oxytocin, where they are thought to reflect increased social approach [24]. Fixation time to the eye region therefore serves as an additional indicator of social response to stress.

We hypothesized that, consistent with the tend-and-befriend model, female participants will show greater prosocial responses following psychosocial acute stress as compared to a non-stressful control condition: their social attributions will be more positive, and they will direct their gaze more at targets' eye region. Male participants, however, will show more anti-social patterns: their attributions will be less positive, and they will direct their gaze away from targets' eye region. In addition, we hypothesized that stress would lead out-group targets to be perceived less positively compared to in-group targets, and that we will observe less gaze to the eye region of outgroup targets, both for female and male participants. Due to the lack of empirical data on the interaction between stress and hormonal status on social responses, we did not specify directional effects here.

2. Material and methods

2.1. Participants

143 university students (71 male) participated in the study (mean age: 24.54 years, SD = 1.97), all study participants were Israeli Jews from Mizrahi and/or Ashkenazi origins. Following the exclusion of 23 participants (11 male) due to malfunctions in eye-tracking calibration or prior acquaintance with the experimenter, the final sample size consisted of n = 120 (60 male). Sensitivity analyses (G*power 3.1.9.2 [25]; indicated that with this sample size we are sufficiently powered to identify 3-way interaction effects (treatment x sex x pre-post) of f = 0.153 or larger. This corresponds to a Cohen's d of 0.31 (small to medium effect size). Exclusion criteria were mental or physical illness, use of medications, wearing eyeglasses, and smoking more than five cigarettes per day. Females with irregular menstrual cycles were also excluded from the study. Female subjects (n = 60) were recruited in three different hormonal statuses. A) Hormonal Contraceptives: 27 females (stress group n = 12) using hormone-based contraceptives, in days 1–21 of their menstrual cycle, i.e., all days except menstruation (see Supplementary Material section 1 for descriptions of contraceptives used). B) Early-Follicular phase: 17 females (stress group n = 7) with a free, regular cycle in days 1–8 of their cycle. C) Mid-Luteal phase: 16 females (stress group n = 9) with a free, regular cycle in days 18–24 of their cycle. Sensitivity analysis indicated that analyses examining group differences as a function of stress x hormone cycle phase are only sufficiently powered for effect sizes of f = 0.24 (medium effect size) or larger. Participants were recruited via the university's online experiment registration system and via social media, and received a fee of 120 NIS (~36 USD) or 12 research credit points in return for their participation.

2.2. Experimental procedure and material

2.2.1. Procedure

Following a telephone screen to ensure participants met study criteria, subjects were randomly assigned to either the Trier Social Stress Test (TSST) or the control treatment (see Section 2.2.4). Female subjects were assigned a date for participation in accordance with their self-reported hormonal status. Females with a free cycle were contacted in the days before their participation to ensure their day in the cycle was aligned with the timing of experimental trials. 72 h prior to testing, subjects filled out an online questionnaire battery (see Section 2.2.2). To limit variance in cortisol levels, subjects were instructed to refrain from excessive physical activity and alcohol for 24 h, and from eating and drinking (besides water) for 120 min prior to the testing session. To control for circadian rhythms in cortisol levels, all experiments commenced at either 14:00 or 16:30. Upon arriving to the lab, subjects provided written informed consent, rested for 20 min, and performed the first session of the facial evaluation and eye tracking task. Subsequently, the TSST/control session was carried out, immediately followed by the second session of facial evaluation and eye tracking task (containing a different set of faces). Two additional tasks were performed by participants unrelated to the current study and will be reported elsewhere. Cortisol levels and psychological stress and anxiety were tested throughout the experiment. At the end of the experiment, participants were debriefed and paid.

2.2.2. Questionnaires battery

Participants were assessed via the following instruments: *Social Phobia Inventory* (SPIN; Connor et al., 2000) to measure social social-related anxiety symptoms; *Beck's Depression Inventory* (BDI; Beck, Steer, & Brown, 1996) to measure depression symptoms; *Interpersonal Reactivity Index* (IRI; Davis, 1983), to assess empathy levels; *Demographic details*, including socioeconomic status and age. TSST and control groups did not differ on their scores for these inventories (see Supplementary Table 1).

2.2.3. Facial evaluation and eye tracking task

Participants were asked to observe and evaluate facial traits in a series of 50 photos of unfamiliar male and female faces, presented in random order. Each photo appeared for 2 s in a full screen mode (1440 × 1080 pixels, covering approximately 24 × 18° of the visual field), then was minimized and moved to the corner of the screen. Participants had a total of 18 s to rate, on a slider from 0 to 100, "how trustworthy/ attractive/ dominant/industrious is this person?". Trustworthiness and attractiveness were chosen as positive social traits, dominance as a trait associated with threat [26], and industriousness as a positive control trait unrelated to the social domain.

Our original dataset of 100 pictures contained faces of males and females with neutral expressions and of different ethnicities in Israel: Israeli Jews of either Mizrahi and/or Ashkenazi ethnicity ("Mizrahi and/ or Ashkenazi Jews"; the "in-group", captures the two main ethnic groups for Jewish Israelis, consisting of Jews from European, Middle east, and North African origins); Jewish Israelis of Ethiopian ethnicity ("Ethiopian Jews"; an "out-group" ethnic minority); and Palestinian Israeli citizens ("Arabs"; an "out-group" minority in a nationality-based conflict with the majority). The dataset included faces of university students, emulating the participants' real-life peers. The pictures were all frontal, colored, and with equal overall luminance. The data set was divided into two blocks of 50, each comprising an equal number of faces of the different groups and sexes. A pilot study showed that the two blocks were balanced with respect to the ratings of different traits.

2.2.3.1. Apparatus. Gaze position was tracked using SMI 250RED (SensoMotoric Instruments Inc, Teltow, Germany), installed on a DELL laptop. Each participant performed a calibration and validation sessions of five points at the beginning of each session. The data analyzed here were obtained from recordings with an average absolute global validation error of less than 1° of visual angle. Recording sample rate was 250hz. Analysis was based on fixations and saccades parceled from the data by the software provided by SMI (BeGaze, SensoMotoric Instruments Inc). Participants were positioned approximately 60 cm from the monitor. The monitor resolution was 1920 × 1080.

2.2.4. Stress induction

Participants underwent the Trier Social Stress Test (TSST; [15], an extensively validated stress procedure shown to elicit a robust cortisol response. The standardized protocol included an anticipatory period (10 min), followed by a public speaking task (5 min) and mental arithmetic task (5 min). The two tasks were performed in front of a panel comprised of female and male evaluators (experimenters wearing lab coats who adopt a non-responsive demeanor). To increase social evaluative threat, participants were informed that their performance is videotaped and that the evaluators are experts in non-verbal coding. The control treatment was designed to follow as closely as possible the TSST, replicating the instructions and time points, however absent the audience and therefore any social evaluative component, and employing a simple mental arithmetic task [27].

2.2.5. Endocrine stress response

Salivary cortisol, a reliable measure of HPA axis reactivity, was collected using Salivette swabs (Sarstedt®, Nümbrecht-Rommelsdorf, Germany). Measurements occurred at 5 time points throughout the experiment: −60, −20, +12, +50, +60 min, relative to stress or control treatment onset. Samples were kept on ice during the session, centrifuged at 3000 rpm at 4 °C for 10 min directly following the experimental task, and stored at −70 °C. Cortisol concentrations were analyzed via a commercially available ELISA kit (Salimetrics), following the manufacturer's instructions. Inter-assay coefficient of variation ranged from 6.1% (high control) to 7.5% (low control), and intra-assay coefficient of variation was 5.3%. Changes in cortisol levels were calculated using Area Under the Curve with respect to increase (AUCi), following the procedures described by Ref. [28].

2.2.6. Subjective stress and anxiety measures

Participants rated current subjective stress and anxiety levels on a scale of 1–100 via a visual analog scale (VAS; "how much stress/ anxiety are you currently experiencing?"). Measurements were performed at three time points (−55, +12, and +65 min, relative to stress onset).

2.3. Statistical analysis

Levels of salivary cortisol and subjective stress and anxiety were used to validate the stress manipulation. A two-way ANOVA model was applied for each stress measure: cortisol levels (measured by Area Under the Curve with respect to increase (AUCi)), subjective stress levels, and subjective anxiety levels. Each model included two factors: treatment (TSST/control) and subject-sex (female/male participants). In addition to the AUCi analysis, we analyzed the cortisol time course. This model included an additional factor of time (5 time-points of cortisol measurements).

Facial evaluation ratings were analyzed using mixed-ANOVA models, with treatment and subject-sex as between-subject factors. The models also included two within-subject factors: session (pre/post treatment) and stimuli-sex (female/male facial target). Importantly, the study focuses on two interaction effects: treatment x session, and treatment x session x subject-sex. To further evaluate how these interactions influence trait ratings, we compared alternative models by computing the Bayes factor. We compared the full model, which included all the factors mentioned above (treatment x subject-sex x session x stimuli-sex) with two partial models: a model which excluded treatment × session interactions, and a model which excluded treatment x session x subject-sex interactions. Bayes factors were computed using the "BayesFactors" package with default parameters [29].

The models of attractiveness and industriousness ratings did not meet the ANOVA normality residuals assumption. To examine possible influence of outliers on these particular models, we examined them after excluding outliers (using thresholds of 3SD and 2.5SD). This procedure did not qualitatively change the results (i.e., no statistical inferences were changed), and so we therefore report models including all ratings.

Next, we examined two additional sets of models. The first set focused on the influence of hormonal status, within female participants, on stress responses. We reanalyzed the facial evaluation ratings with female participants' data only. This allowed us to add a between-subject factor to capture the hormonal status of each female participant (hormonal contraceptives, early-follicular phase and mid-luteal phase). The second set of models tested female and male data with subject-sex as a between-subjects factor. However, in contrast to the previous models, we now included a group membership factor, to capture the social group of each presented face (Mizrahi and/or Ashkenazi Jews, Ethiopian Jews, and Arabs). We did not include this factor in previous models because the addition of the factor results in violation of the model assumption (see further details in results, Section 3.4).

Finally, we used mixed-ANOVA models to investigate the tendency to look at the eye regions as a result of stress exposure. We applied the same models we used in the first facial evaluation ratings analysis for the complete sample, female and male, only now with the gaze time to the eye-region as the dependent variable.

In cases of heterogeneity of covariance (Mauchly test of sphericity), we determined statistical significance after applying Greenhouse-Geisser corrections. Following significant interaction effects, we performed contrasts analysis using "Tukey" p-values adjustments. We report η_p^2 values for all effects. We analyzed the data using R-Studio's packages "lmer" and "BayesFactor" [29,30].

3. Results

3.1. Validation of stress induction procedure

We used levels of salivary cortisol, as well as subjective stress and anxiety measures, to validate that acute stress manipulation caused an increase in the physiological and psychological stress response.

3.1.1. Salivary cortisol levels

As in previous studies, the TSST procedure successfully increased participants' salivary cortisol levels. A two-way ANOVA was conducted on cortisol levels. The model included treatment (stress/control) and subject-sex (female/male) as factors. We found the expected treatment × session interaction ($F(2.16, 250.4) = 23.47$, $p < 0.001$, $\eta_p^2 = 0.17$), whereby cortisol levels increased following manipulation onset in the TSST treatment but not in the control treatment. As shown in Fig. 1 (Panel A), the model also revealed a significant interaction between treatment, session, and subject-sex ($F(2.16, 250.4) = 4.04$ $p = 0.02$, $\eta_p^2 = 0.034$). Interactions between sessions and treatment statistics values were corrected using Greenhouse-Geisser correction (see methods).

In line with previous studies, males showed a significantly higher cortisol increase compared to females [31]. Similar findings were observed when examining AUCi ($F(1,116) = 40.46$, $p < 0.001$, $\eta_p^2 = 0.26$), whereby cortisol levels increased following manipulation onset in the TSST treatment but not in the control treatment. As shown in Fig. 1 (Panel B), the model also revealed a significant interaction between treatment and subject-sex ($F(1,116) = 7.25$, $p = 0.008$, $\eta_p^2 = 0.06$).

3.1.2. Subjective stress and anxiety levels

Similar to salivary cortisol levels, subjective stress and anxiety levels were increased by the TSST treatment, but not by the control treatment. A two-way ANOVA was conducted on the subjective stress and anxiety levels. The model included treatment (stress/control) and subject-sex (female/male) as factors. We found a significant treatment effect for both stress ($F(1,116) = 15.24$, $p < 0.001$, $\eta_p^2 = 0.12$) and anxiety ($F(1,116) = 12.72$, $p < 0.001$, $\eta_p^2 = 0.1$) (see Fig. 1, Panels C and D). The main effect and interaction that include subject-sex were not significant, reflecting a similar subjective stress response in females and males.

3.2. Facial trait evaluation

Ratings of trustworthiness, attractiveness, dominance, and industriousness are presented in Table 1. For each trait rating, we applied a mixed ANOVA model with two between-subjects factors and two within-subject factors. The between-subject factors were treatment (TSST/control) and subject-sex (female/male). The within-subject factors were

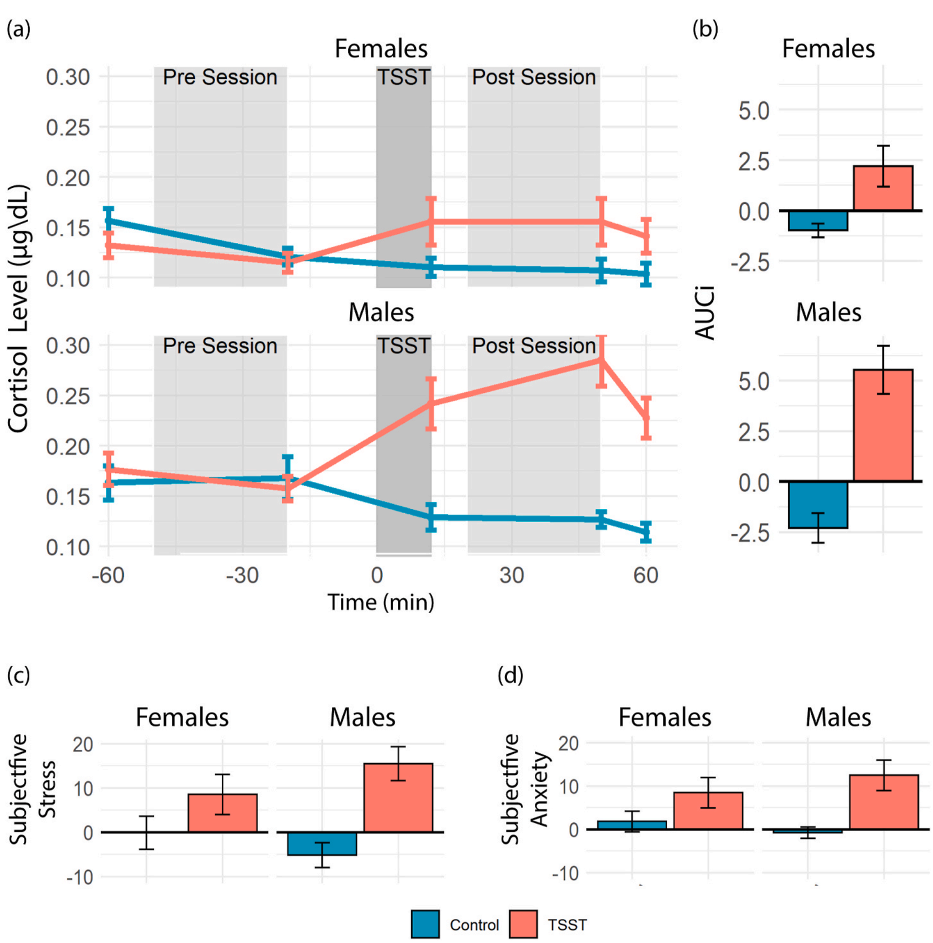

Fig. 1. Validation of acute stress induction – females and males. (a)Salivary cortisol levels throughout the experiment, (b) Area under the curve of cortisol levels (AUCi), (c) difference in subjective ratings of stress between post- and pre-manipulation, (d) difference in subjective ratings of anxiety between post- and pre-manipulation. Red and cyan represent the stress manipulation group and controls, respectively. The vertical lines represent the standard errors across participants. The time, in minutes, is relative to the manipulation onset, defined as 0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Statistics of the facial evaluation task – females and males. The table includes the statistical results of the ANOVA models of all attributions (Trustworthiness, Attractiveness, Dominance, and Industriousness). Stress-related effects are presented in the rows labeled treatment x session, and significant results are present in bold. The table contains the main effects and stress-related interactions. The full statistical model for each trait, including effects unrelated to stress, is displayed in the Supplementary Material (Tables 2–5, 8).

	Trustworthiness			Attractiveness			Dominance			Industriousness		
	F	p	η_p^2	F	P	η_p^2	F	p	η_p^2	F	p	η_p^2
Treatment (TSST/control) F(1,116)	1.422	0.24	0.01	0.45	0.5	<0.01	0.01	0.94	0.00	0.04	0.83	<0.01
Session (pre/post manipulation) F(1,116)	0.76	0.39	0.01	**6.62**	**0.01**	**0.05**	0.15	0.70	<0.01	0.28	0.60	<0.01
Subject-sex (female/male) F(1,116)	2.84	0.1	0.02	0.3	0.89	<0.01	<0.01	1.00	<0.01	1.03	0.31	0.01
Treatment * session F(1,116)	1.25	0.27	0.01	0.48	0.49	<0.01	3.39	0.07	0.03	3.52	0.06	0.03
Treatment * session * subject-sex F(1,116)	1.6	0.21	0.01	0.7	0.41	0.01	0.97	0.33	0.01	0.33	0.57	<0.01
Stimuli-sex (female/male) F(1,116)	**198.46**	**< 0.001**	**0.63**	**99.57**	**< 0.001**	**0.46**	1.42	0.24	0.01	**239.94**	**< 0.001**	**0.67**
Subject-sex * Stimuli-sex F(1,116)	0.53	0.47	0.01	**11.89**	**< 0.001**	**0.09**	2.08	0.15	0.02	<0.01	0.99	<0.01

session (pre/post manipulation) and stimuli-sex (male/female). Stress-related effects, the focus of this study, are presented in Table 1.

As in previous studies [7], our models revealed stimuli-sex main effects in trustworthiness and attractiveness, where ratings for females' faces were significantly higher in comparison to males (trustworthiness: $F(1,116) = 198.46$, $p < 0.001$, $\eta_p^2 = 0.63$; attractiveness: $F(1,116) = 99.57$, $p < 0.001$, $\eta_p^2 = 0.46$). In industriousness too, we found higher ratings for females compared to males ($F(1,116) = 239.94$, $p < 0.001$, $\eta_p^2 = 0.67$). We did not find stimuli-sex effects for dominance ratings. We also found higher attractiveness rating in the post session compared to the pre session ($F(1,116) = 6.62$, $p = 0.01$, $\eta_p^2 = 0.05$). We did not find time effects for the other rating scales.

As described in Table 1, no stress-related effects were found to be significant for any of the ratings scales. To test whether effects were present when examining individual changes in cortisol reactivity, we applied alternative classification methods. First, we examined differences between cortisol responders and non-responders by applying the classification thresholds proposed by Ref. [33]. Second, we replaced the binary treatment effect (TSST/control) with a continuous measure of AUCi.

Applying the responders vs. non-responders cutoffs did not meaningfully change our results (i.e., statistical inferences regarding significance remain unchanged), additional details are provided in the Supplementary material, Section 4. When applying the continuous AUCi measure, results remained non-significant for attractiveness and dominance ($p > 0.1$). However, we found a significant 4-way interaction (AUCi x session x subject-sex x stimuli-sex), for trustworthiness ($F(1,116) = 6.37$, $p = 0.013$) and industriousness ($F(1,116) = 4$, $p = 0.047$). To further explore these effects, we analyzed male and female participants' ratings separately. Only the females' trustworthiness model revealed a significant 3-way interaction of treatment x session x stimuli -sex ($F(1,58) = 6.31$, $p = 0.015$). Meaning, comparing to control treatment, stress treatment decreased females' trustworthiness ratings toward female faces only.

One method for examining the non-significant stress-related findings is the use of Bayesian statistics to assess the probability that the data are more in support of models including stress-related effects on facial attributions, or more in support of models excluding stress-related effects. We compared three models (i.e., hypotheses): H_1, the full model (including treatment, subject-sex, session, stimuli-sex); H_2, the full model without the interactions with treatment x session; and H_3, the full model without the interactions with treatment x session x subject-sex. Then, we computed two Bayes Factors: BF_{12} (comparing H_1 and H_2) indicating how more likely is H_1 compared with H_2, and BF_{13} (comparing H_1 and H_3) indicating how more likely is H_1 compared with H_3. Results are displayed in Table 2. Models without stress-related effects (H_2 and H_3) were more likely to explain the data compared to models including stress-related effects (H_1). Namely, stress exposure, as well as the sex of the participant, did not predict trait attributions (while the sex of the observed face did).

3.3. Facial trait evaluation and females' hormonal status

Next, we examined whether females' hormonal status interacts with stress exposure to influence facial evaluation ratings. For each trait, mixed-ANOVA models were applied. The between-subject factors were treatment (TSST/control) and hormonal status (hormonal contraceptives, early-follicular phase and mid-luteal phase). The within-subject factors were session (pre/post manipulation) and stimuli-sex (male/female) (see Table 3). The full statistical model for each trait, including effects unrelated to stress, is displayed in the Supplementary Material (Tables 2–5).

The analysis revealed significant stress-related interactions in trustworthiness, attractiveness, and dominance (see Fig. 2 and Table 3). For trustworthiness, a significant interaction between treatment and session was found. Importantly, this effect was moderated by hormonal status: reflected as a significant 3-way interaction between treatment, session, and hormonal status. Post hoc contrasts tests (using Tukey method for p-value adjustments) revealed that stressed female participants in the early-follicular phase rated faces as less trustworthy compared to control female participants in the same phase: early-follicular phase participants in the control treatment ($t(54) = -3.62$, $p = 0.002$), females using hormonal contraceptives in both TSST and control treatments ($t(54) = -0.69$, $p = 0.87$), and mid-luteal phase females in both TSST and control treatments ($t(54) = 0.59$, $p = 0.91$).

Analysis of attractiveness scores revealed a marginal significant increase in ratings in females in general, and, importantly, a significant 3-

Table 2

Bayes Factors comparing models with and without the session × treatment interactions –females and males. The table includes the Bayes Factors (BF) for comparing the full model (H_1), which include the treatment × session interaction (stress-related interaction), to a model without the treatment × session interaction (H_2) and to a model without the treatment x session x subject-sex interaction (H_3). The Bayes Factors were computed for each attribute separately.

	Trustworthiness	Attractiveness	Dominance	Industriousness
$BF_{1,2}$	0.01	<0.01	0.02	0.01
$BF_{1,3}$	0.16	0.08	0.08	0.05

Table 3
Statistics of the facial evaluation task – females only. The table includes the statistical results of the ANOVA models of all attributions (trustworthiness, attractiveness, dominance, and industriousness). Stress-related effects (treatment x session) rows are highlighted, and significant results are present in bold. The table contains main effects and stress-related interactions. The full statistical model for each trait, including effects unrelated to stress, is displayed in the Supplementary Material (Tables 2–5).

	Trustworthiness			Attractiveness			Dominance			Industriousness		
	F	P	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2
Treatment (TSST/control) F(1,54) =	1.65	0.21	0.03	2.29	0.14	0.04	0.87	0.36	0.02	0.03	0.86	<0.01
Session (pre/post) F(1,54) =	0.01	0.94	<0.01	3.71	0.06	0.06	0.09	0.77	<0.01	0.35	0.56	0.01
Hormonal status F(2,54)	1.89	0.016	0.07	0.85	0.43	0.03	1.34	0.27	0.05	1.25	0.30	0.04
Treatment * session F(1,54)	**4.79**	**0.03**	**0.08**	2.05	0.16	0.04	**4.97**	**0.03**	**0.08**	3	0.09	0.05
Treatment * session * Hormonal status F(2,54	**4.84**	**0.012**	**0.15**	**3.58**	**0.04**	**0.12**	0.56	0.57	0.02	1.05	0.36	0.04
Stimuli-sex F(1,54)	**72.73**	**< 0.001**	**0.57**	**122.99**	**< 0.001**	**0.7**	<0.01	0.99	<0.01	**123.97**	**< 0.001**	**0.70**
Hormonal status * Stimuli-sex F(2,54)	1.19	0.31	0.04	1.24	0.30	0.04	0.33	0.72	0.01	0.77	0.47	0.03

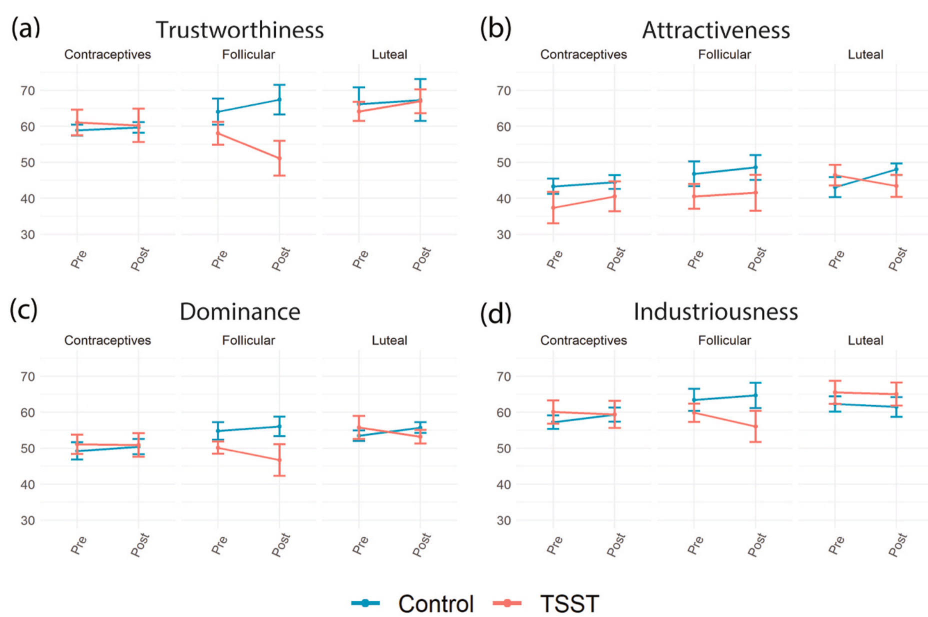

Fig. 2. Facial trait evaluation ratings by hormonal status – females only. Attributes' ratings in pre and post manipulation sessions of each hormonal status group. Red and cyan represent the stress manipulation group and controls, respectively. The vertical lines represent the standard errors across participants. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

way interaction of session, treatment, and hormonal status. Post hoc Tukey tests revealed stress-related decrease of attractiveness ratings in mid-luteal phase participants ($t(54) = -2.69$, $p = 0.028$), but not in the hormonal contraceptives participants ($t(54) = 0.89$, $p = 0.78$) and early-follicular phase participants ($t(54) = -0.26$, $p = 0.99$). Meaning, despite a mild increase in attractiveness ratings in all females, stressed mid-luteal phase participants rated others as less attractive.

For dominance ratings, the model revealed a significant interaction between treatment and session ($F(1,54) = 4.97$, $p = 0.03$, $\eta_p^2 = 0.084$), reflecting a moderate decrease in all females' groups in dominance ratings after stress, in contrast to a mild increase in the control treatment.

We did not observe any significant stress-related effects for Industriousness.

3.4. In-group vs. out-group

As mentioned, the current study stimuli were comprised of faces from three different social groups in Israel: Mizrahi and/or Ashkenazi Jews, Ethiopian Jews, and Arabs. In general, we found main effects of group membership across different traits, reflecting a considerable outgroup bias against Ethiopian Jews and/or Arabs. A separate pilot test (n = 80 females and males) showed that when asked to do so, individuals were able to distinguish ethnic identities between Mizrahi and/or Ashkenazi Jews and Arabs faces (see Supplementary material section 5). The group membership factor was not included in the traits analyses mentioned in previous sections due to large differences in within-group variance and violation of model assumptions (normality of residuals and significant differences in the variance of the residuals across social groups). However, as an exploratory analysis, in this section we did include group-membership as a within-subject factor in our models (in addition to treatment, subject-sex, time and stimuli-sex). We did not find a significant interaction between treatment, session (pre-vs.

post stress) and group membership (see Fig. 3. For further details, see Supplementary material section 6). Meaning, for example, that stress did not differentially affect trustworthiness rankings of the different social groups.

3.5. Eye movements patterns

To examine the percent of fixation time on the eyes, we applied a mixed ANOVA model for both females and males. The model included two between-subject factors: treatment (TSST/control), and subject-sex (female/male), and two within-subject factors of session (pre/post manipulation) and stimuli-sex (female/male). Stress did not yield significant effects (treatment x session: $F(1,107) = 0.18$, p 0.67, $\eta_p^2 < 0.01$); treatment x session x subject-sex: $F(1,107) = 0.13$, $p = 0.72$, $\eta_p^2 < 0.01$). Thus, stress did not affect the amount of time in which participants fixated on the eyes of faces. All other effects, such as stimuli-sex, were not significant as well (see Supplementary Material Table 7). Importantly, as in previous studies (for example [34,35], the model revealed high intra-class-correlation (ICC) for fixation time on the eyes at the participant level (ICC = 0.96), which highlights the high amount of variance explained solely by the participant identity.

In an exploratory analysis, using linear regression, we examined whether the fixation time on the eyes (as an independent variable) can predict the attributes' ratings (as a dependent variable), when considering subject-sex and stimuli-sex as additional factors. This revealed, in addition to the influence of subject-sex and stimuli-sex, a significant interaction between subject-sex and fixation time on the eyes in trustworthiness ($t(137.34) = -2.17$, $p = 0.032$) and industriousness ($t(130.17) = -2.5$, $p = 0.014$). Both interactions suggest a positive relation between higher ratings and time the participants look at the eye regions in male participants, compared to no relation in female participants.

4. Discussion

The current study examined the effects of acute psychosocial stress on social attributions and eye movement patterns. In general, our findings show that acute psychosocial stress exposure did not affect these socio-cognitive functions, even in the case when targets belonged to different social groups. However, different hormonal statuses in females interacted with stress exposure to yield idiosyncratic social outcomes.

Participants, in general, did not rate faces as either more or less trustworthy, attractive, dominant or industrious following stress exposure. Notably, these largely null findings are evident without correction for multiple testing and are further corroborated by our Bayesian analysis; all models without treatment-by-session interactions better explained our findings, as compared to models including these interaction terms. Likewise, we did not find support for our hypothesis on an effect of acute stress on eye movement patterns. Specifically, participants did not fixate more or less into targets' eye region following stress exposure. Our results therefore do not coincide with recent publications suggesting that acute stress facilitates prosocial responses (for example

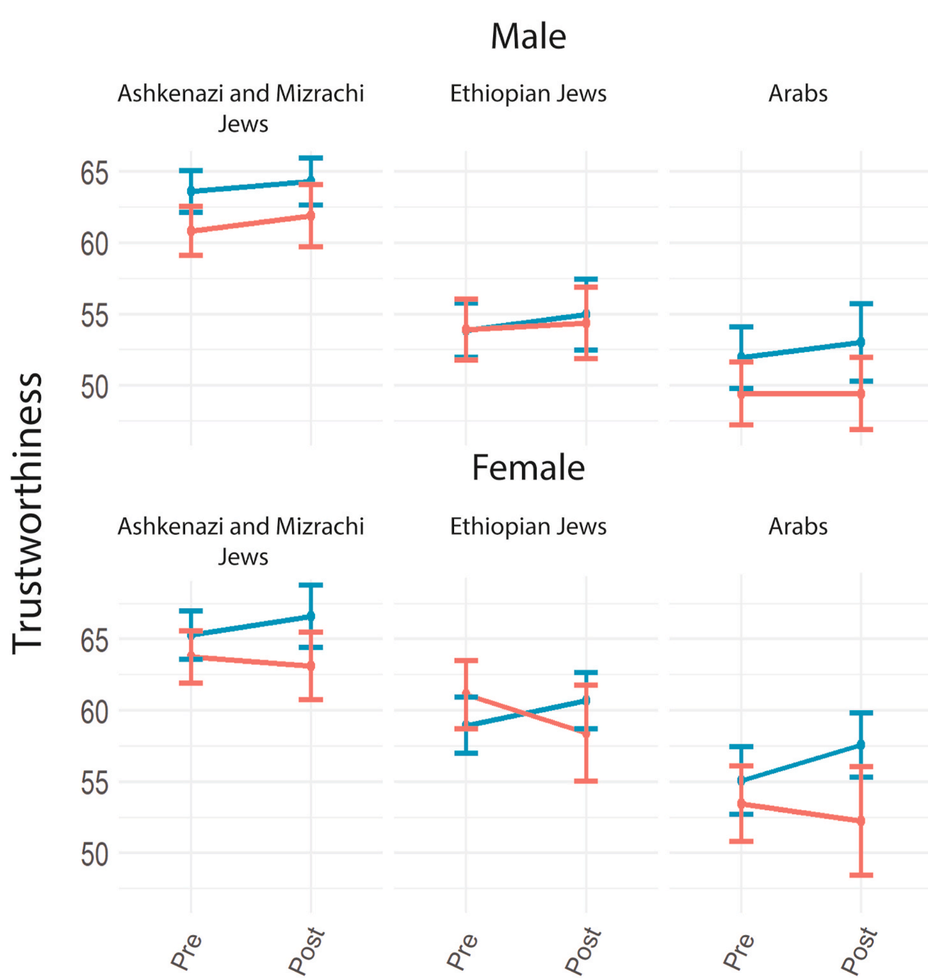

Fig. 3. Facial trait evaluation ratings by targets' social groups – females and males. Trustworthiness ratings in pre and post manipulation sessions. Results presented separately for each social group. Red and cyan represent the stress manipulation group and controls, respectively. The vertical lines represent the standard errors across participants. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

[36], or antisocial responses (for example, [37]. Rather, they are consistent with studies showing that acute stress induced by the same paradigm as in the current study (one-person TSST) does not affect everyday moral decision-making [11] or other-regarding preferences [38].

Notably, we did find considerable differences in baseline ratings between different ethnic groups, validating that our facial attributions task succeeded in eliciting significant differences in ratings between in-group and out-group members. For example, participants' average trustworthiness ratings for Arab targets was 10.9 (out of 100) points lower than that of Mizrahi and/or Ashkenazi Jewish targets (Cohen's d: 1.03, a large effect size); For Ethiopian Jews faces, the average trustworthiness rating was 8.7 points lower than that of Mizrahi and/or Ashkenazi faces (Cohen's d: 0.66, a medium effect size). Nevertheless, acute stress did not differentially affect social attributions ratings or eye gaze as a function of targets' group membership. These null stress-related effect is inconsistent with a recent paper that showed males perceived out-group members as less trustworthy following acute stress manipulation [19]. The different findings may be due to the context of the stressor. As opposed to the current study [19], did not use the TSST, but a within-subject version of the Maastricht Acute Stress Test (MAST; [39]. The original MAST protocol compares participants in the stress group (where participants immerse their hand in ice-water while performing a demanding mental arithmetic task in front of an experimenter and a video camera) and participants in the control group (immersing their hand in lukewarm water while performing a simple mental arithmetic task, when an experimenter is present in the room). The participants in Ref. [19] study, however, underwent both control and stress conditions, separated by a rest period. The different stress paradigms, and the alternating within-subject design in Ref. [19]; may have contributed to the contrasting results (see, for example [40], meta-analysis on the effects of alternations in stress induction procedure in the TSST). Another possible explanation to the different results concerns the nature of the ethnic groups in the studies. As opposed to the groups tested in the current study (Mizrahi and/or Ashkenazi Jews, Ethiopian Jews and Arabs) [19], groups consisted of Whites, Arabs, Blacks, and Southeast-Asians. It was previously found that different out-groups elicit different emotions and threat levels, suggesting that general measures of intergroup bias may mask specific group-based differences [41]. Importantly, our findings are supported by another large-scale study, which included both female and male participants. The study found that despite being given the opportunity to exhibit pro/anti-social behaviors towards the in-group and the out-group, participants showed no main effects of acute stress on intergroup resource allocation [42].

Interestingly, we did find robust psychosocial stress effects on social attributions made by females in different hormonal statuses. Previous studies have highlighted the role of hormonal status in contributing to ratings of facial features, such as attractiveness [43], as well as other outcomes, such as memory, learning and attention [44]. Here we find that stress impacted the attributions as a function of females' hormonal status; however, rather than being in a specific direction, the effects were idiosyncratic and trait-specific. We found that stressed females in the early-follicular phase rated others as less trustworthy; stressed females in the mid-luteal phase perceived other as less attractive; and stressed females of all hormonal statuses (hormonal contraceptives, early-follicular phase and mid-luteal phase) found others to be less dominant. Industriousness ratings were unaffected by stress, which confirms that our stress exposure specifically affected social attributions and not all traits. Specifically, in regards to trust, which plays a key role in social relations [45] and stress [46]. We showed that following stress exposure, trust levels dropped only in females in the early-follicular phase but not in any of the other groups. The early-follicular phase is associated with more negative mood [47,48]; which prior studies have shown has a detrimental effect on trust [49]. One possible mechanism accounting for these findings may be the anxiolytic and sedative effects associated with progesterone (see Ref. [50]; indeed, the early-follicular phase is characterized by lower levels of progesterone [51]. Taken together with our findings of idiosyncratic effects on other social attributions (attractiveness, dominance), the current study depicts a complexed, multifaceted picture of acute stress effects on female social attributions. These influences depend on specific hormonal statuses as well as perceived social traits, which points to great flexibility granted by the female hormonal system, presumably allowing for adjusting responses to everchanging circumstances, challenges and demands.

Our study sample for each hormonal status group was small, and we are also aware that the field of females' hormonal status and sociality has a long history of findings that failed to replicate (see Ref. [52]. We therefore suggest that until replicated, these hormonal status-dependent stress effects should be treated with caution. Nevertheless, our findings are in line with the literature showing that different hormonal statuses modulate females' responses. These effects, which have been linked to fluctuating levels of the ovarian hormones estrogen and progesterone, are known to alter acute stress responses (for example [51], as well as social outcomes (for example, [53]. We elaborate on this literature by showing that under stressful circumstances, the effects of hormonal status extend to the initial process of perceiving of others and attributing them with different social traits.

Our study presents some limitations. We examined the social traits trustworthiness, attractiveness and dominance because research has highlighted these as being central [26]. However, other traits, that are more theory-specific, may induce different social outcomes following stress exposure (for example, Hostility to test fight-or-flight, Approachability to test tend-and-befriend). Also, our measures of hormonal status were based on self-reports. Objective measures of hormonal concentrations in plasma could enable more precise discrimination between hormonal statuses. Finally, almost all studies to date, as well as ours, have examined social responses to stress with others who are anonymous or not previously known. It may be that social responses towards friends and/or family may show a different pattern of results.

From an evolutionary psychology perspective, our mixed findings on social attributions and eye movement patterns challenge elements of both leading theories in the field of stress effects on sociality. We found no support for tend-and-befriend patterns in either females or males. Similarly, fight-or-flight tendencies were not evident for males. Surprisingly, in females – theorized to affiliate with others under stressful circumstances – we find some sort for fight-or-flight with different hormonal statuses interacting with stress to cause trait-specific anti-social responses. These results suggest that further research is needed in order to refine and specify how psychosocial stress affect different social outcomes.

The current study, taken together with previous mixed findings in the field of stress and sociality, point to a few important future directions. First, larger sample sizes of female and male participants may be required to assess acute stress effects on social responses. This is specifically critical in the case of female participants, which needs to be divided to hormonal status-based subgroups. Second, more systematic research should take place whereby the one-person vs. group versions of the TSST are manipulated within the same study, to understand the specifics of the stress context on social effects. First steps towards this important goal were taken recently [54]. Third, additional measurements of stress reactivity (physiological and psychological) would allow for a more fine-grained analyses of how these processes may affect social attributions. Fourth, there is a need for greater research examining different facets of sociality. This will help elucidate if the last decade of inconsistent findings reflects a theoretical phenomenon rather than a methodological issue, as some aspects of social cognition or behavior might be more (or less, or not at all) susceptible to psychosocial stress effects than others.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100093.

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