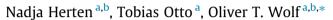
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The role of eye fixation in memory enhancement under stress – An eye tracking study $\stackrel{\mbox{\tiny $\%$}}{}$



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ABSTRACT

In a stressful situation, attention is shifted to potentially relevant stimuli. Recent studies from our laboratory revealed that participants stressed perform superior in a recognition task involving objects of the stressful episode. In order to characterize the role of a stress induced alteration in visual exploration, the present study investigated whether participants experiencing a laboratory social stress situation differ in their fixation from participants of a control group. Further, we aimed at shedding light on the relation of fixation behaviour with obtained memory measures. We randomly assigned 32 male and 31 female participants to a control or a stress condition consisting of the Trier Social Stress Test (TSST), a public speaking paradigm causing social evaluative threat. In an established 'friendly' control condition (f-TSST) participants talk to a friendly committee. During both conditions, the committee members used ten office items (central objects) while another ten objects were present without being used (peripheral objects). Participants wore eye tracking glasses recording their fixations. On the next day, participants performed free recall and recognition tasks involving the objects present the day before. Stressed participants showed enhanced memory for central objects, accompanied by longer fixation times and larger fixation amounts on these objects. Contrasting this, fixation towards the committee faces showed the reversed pattern; here, control participants exhibited longer fixations. Fixation indices and memory measures were, however, not correlated with each other. Psychosocial stress is associated with altered fixation behaviour. Longer fixation on objects related to the stressful situation may reflect enhanced encoding, whereas diminished face fixation suggests gaze avoidance of aversive, socially threatening stimuli. Modified visual exploration should be considered in future stress research, in particular when focussing on memory for a stressful episode.

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1. Introduction

Stress is known to influence memory processes via activation of sympathetic nervous system and hypothalamus-pituitary-adrenal axis and the related release of cortisol and (nor)adrenaline. As stress exerts an influence on attention (Chajut & Algom, 2003), its effect on memory is already initiated at an early perceptual stage by different attentional mechanisms. During a stressful situation, activation of visual areas is amplified (Henckens, Hermans, Pu, Joels, & Fernandez, 2009), reflecting enhanced processing of relevant information. Furthermore, in studies with electroencephalography or

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magneto encephalography, stress was found to enhance early indices of exogenous attention (N1 and N1m, respectively; Elling et al., 2012; Shackman, Maxwell, McMenamin, Greischar, & Davidson, 2011). This suggests that stress alters early attentional processing, such that memory for items perceived is enhanced. In a stressful situation, attentional narrowing directs the focus towards salient items (see for review: Christianson, 1992). This causes a facilitation of objects relevant during the stressful situation to be processed and stored in long-term memory. Studies investigating stress effects on memory have found that objects of relevance within the stressful situation are remembered better than less significant objects as well as better relative to a nonstressful situation (Christianson, 1992; Echterhoff & Wolf, 2012; Mather & Sutherland, 2011; Wiemers, Schoofs, & Wolf, 2012). It has been suggested that cortisol in interaction with negative affect and arousal mediate this effect (e.g. de Quervain, Aerni, Schelling, & Roozendaal, 2009; Lupien & McEwen, 1997; Quas, Yim, Edelstein,





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Cahill, & Rush, 2010; Roozendaal, McEwen, & Chattarji, 2009; van Ast et al., 2013; Wolf, 2009). Hereby, both effects of attentional processes at encoding and subsequent consolidation processes contribute to memory enhancement under stress (Quas, Rush, Yim, & Nikolayev, 2014). Glucocorticoids, increased through activity of the HPA axis, can bind to glucocorticoid and mineralocorticoid receptors, thereby exerting rapid non-genomic as well as slower genomic actions (Herman, Patel, Akil, & Watson, 1989; Joëls, Fernandez, & Roozendaal, 2011; van Steensel et al., 1996). Their action on hippocampus and amygdala forms the basis of the beneficial effects of stress on encoding and consolidation (Henckens et al., 2009; Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Lupien et al., 2002). While studies could demonstrate selective attention (Chajut & Algom, 2003) and underlying executive functions (Beste, Yildiz, Meissner, & Wolf, 2013; Weerda, Muehlhan, Wolf, & Thiel. 2010) to improve under acute stress, others reported an impairment of these processes (Arnsten, 2009; Plessow, Kiesel, & Kirschbaum, 2012; see for a meta-analysis: Shields, Sazma, & Yonelinas, 2016). Conceivably, stress leads to rather stimulus driven processing with less top-down control of the prefrontal cortex (Arnsten, 2009; Sänger, Bechtold, Schoofs, Blaszkewicz, & Wascher, 2014), whereas bottom-up processing of stimulus features like salience (Buschman & Miller, 2007) dominate in attentional selection and enhance memory for these stimuli (Mather & Sutherland, 2011; Sutherland & Mather, 2012).

Modified attentional processes under stress are often investigated in connection with memory effects. As attention is closely associated with visual processes, fixation measures are conceivable factors influencing memory within a stressful situation, but they have not yet been investigated. Effects of visual exploration can particularly impact memory encoding. For studies comparing the influence of stress on memory encoding with memory consolidation (e. g. Klemfuss, Milojevich, Yim, Rush, & Quas, 2013), visual exploration and fixation measures might thus especially be important variables to consider. Not only fixation, but also visual attention is necessary for binding of sensory features into coherent object representations (Rensink, 2000a,b). Before the object representations can become lasting memories, they are maintained in visual short-term memory where they are stable across brief disruptions and have the potential of being transferred into longterm memory (McGaugh, 1966). In contrast, unattended sensory representations decay rapidly and are overwritten by new visual inputs, despite having been initially fixated (Rensink, 2000a). Dependent on salience and role (central versus peripheral) of the objects within the stressful situation, their representations in short-term memory become consolidated into long-term memory (Kensinger, 2009; Wiemers, Sauvage, Schoofs, Hamacher-Dang, & Wolf, 2013; Yonelinas & Ritchey, 2015). Fixation and focal attention are thus both involved in visual memory encoding and transfer of memory content into long-term memory.

In previous studies investigating memory for objects used by committee members of the Trier Social Stress Test (TSST), which are referred to as central objects (versus peripheral = static objects), we observed enhanced object recognition for central items in the stress group (Wiemers et al., 2013). Comparing fixation behaviour of stressed and control participants in the current study, we assessed its possible association with memory by means of two different tasks – a free recall as well as the aforementioned object recognition task.

As previously described, objects of potential relevance in the stressful situation are remembered better (Christianson, 1992; Echterhoff & Wolf, 2012; Mather & Sutherland, 2011; Wiemers et al., 2012) and are especially attended to. In the TSST, stress is mainly elicited by the committee members due to their cold and reserved behaviour. Thus, items used by them might be or become salient during the stressful episode, even though not relevant to

the situation from the very beginning. Since the committee members are the stress inducing source of social evaluative threat and thus perceived as aversive by the participants, gaze towards them is more likely to be avoided. Moreover, participants of the TSST are prone to feel embarrassed and ashamed which has been associated with gaze avoidance (Edelmann & Neto, 1989). Hence, even though social stimuli like faces are highly salient and contain important information beyond the contents of the interaction (Bahrick & Lickliter, 2014; Caulfield, Ewing, Bank, & Rhodes, 2016; Crivelli, Jarillo, Russell, & Fernández-Dols, 2016), stress is predicted to lead to reduced face fixation. However, the neutral items which are used by the committee are believed to become associated with the main stressor and thus central, potentially meaningful, to the stressful situation.

We hypothesised that under acute stress, fixation behaviour will be characterised by more frequent and longer total fixation and mean fixation times of the objects present in the modified version of the TSST, in particular with regard to central objects which had been used by the committee. Furthermore, we predict these fixation patterns to be related to enhanced memory performance on the next day, assessed by free recall and object recognition tasks.

2. Methods

2.1. Participants

We tested 63 non-smoking male (n = 32) and female students from the Ruhr-University Bochum, none of whom reported psychological or physiological diseases. Women were taking hormonal contraceptives (restricted to monophasic preparations with an ethinylestradiol (0.02–0.035 mg) and a gestagenic component) and were only tested during their pill intake phase (Merz et al., 2012). None of the participants reported regular medication use. The age of the participants tested ranged from 18 to 34 years (M = 23.63, SD = 3.8) and their BMI ranged from 18.75 to 28.23 kg/m² (M = 22.93, SD = 2.53). They were paid an expense allowance of 15 ϵ or received course credits for participating. The study was approved by the local ethic committee of the Faculty of Psychology and the Declaration of Helsinki was followed.

2.2. Experimental session

Participants were randomly assigned to a stress or control condition. First, they signed informed consent and then filled out the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988). For stress induction we used a modified version of the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993; Wiemers et al., 2013). The control condition consisted of the friendly version of the TSST (f-TSST; Wiemers et al., 2012). Shortly before being led into the testing room, a baseline saliva sample (-1 min) was collected by means of a Salivette[®] (Sarstedt, Nümbrecht, Germany) in a preparation room. Before a 5 min preparation time for either of the conditions started, the eye tracking glasses were adjusted to the participant and calibrated by the experimenter (see Section 2.3.4). The experimenter then left the testing room which marked the onset of the preparation phase. After 5 min, the participant started the 10 min speech. When the experimenter returned after these 15 min had passed, she removed the eye tracker and led the participant back to the former preparation room where another saliva sample (+1 min) was collected. Participants again filled out the PANAS and then engaged in a computerised game of dice task, which is not topic of the current report. They delivered the last saliva sample (+20 min), and were debriefed (see Fig. 1 for experimental timeline).

On the second day, participants again filled out the PANAS and delivered a saliva sample to control for baseline cortisol concentration (pre). Afterwards, the experimenter asked participants for a free recall of the 20 objects present in the testing room. Then, participants engaged in an object recognition task on the computer and delivered the final saliva sample (post).

2.3. Material

2.3.1. Stress procedure and control condition

2.3.1.1. Trier Social Stress Test (TSST). Stress was induced using the TSST (Kirschbaum et al., 1993). The TSST reliably activates the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis (Dickerson & Kemeny, 2004). In the paradigm, socioevaluative threat and uncontrollability lead to a stress reaction. The participants are asked to imagine to be applying for a job position in front of a committee by only referring to their character traits. The common pursuit to succeed in a job interview leads to intrinsic motivation to perform well. The committee, consisting of one female and one male, are introduced as trained behavioural psychologists analysing the participants' behaviour, occasionally taking notes. Since they act very reserved, the participant does not receive any feedback for his performance which is perceived as cold, reserved and negative. The situation is videotaped which adds to the feeling of being evaluated. In line with Wiemers et al. (2013), the mental arithmetic task was omitted in favour of the speech being extended to ten minutes.

2.3.1.2. Friendly-TSST (f-TSST). The recently established control condition, the f-TSST, does not activate the HPA axis. Affect ratings show that also subjectively participants do not experience the control condition as stressful (Wiemers et al., 2013). Here, the committee members are introduced by their names, friendly interacting with the participants. The participants are not videotaped and can choose from a set of topics comparable to the contents of a job interview. Except for the mentioned, the procedure is identical to the TSST.

2.3.2. Physiological stress measures

2.3.2.1. Salivary cortisol. Participants were instructed to refrain from taking medication or other drugs, drinking alcohol or engaging in excessive sports one day, and from drinking anything except water and brushing their teeth one hour before testing. On the first day, three and on the second day, two saliva samples were collected using Salivettes[®]. Samples were deep-frozen at –18 °C and analysed using a Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA), as described elsewhere (Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The detection limit for salivary cortisol was set at 0.5 nmol/L. Intra- and inter-assay coefficients of variation were below 13%. To account for the circadian rhythm of cortisol release, testing took place always at the same time window, in between 9:00 am and 12:30 pm.

2.3.2.2. Salivary alpha amylase. In addition, the enzyme alphaamylase (sAA) was analysed from the saliva samples for assessing the response of the sympathetic nervous system (Rohleder & Nater, 2009). A colorimetric test using 2-chloro-4-nitrophenyl- α -maltro triosoide (CNP-G3) as a substrate reagent was applied to measure sAA concentration (Lorentz, Gütschow, & Renner, 1999; Winndeen, David, Sigier, & Chavez, 1988). Intra- and inter-assay variabilities were below 10%.

2.3.3. Affect measurements

The Positive and Negative Affect Scale (PANAS; Watson et al., 1988) including 20 items, 10 expressing positive and 10 negative emotions, was used for participants' affect ratings. On a 5-point

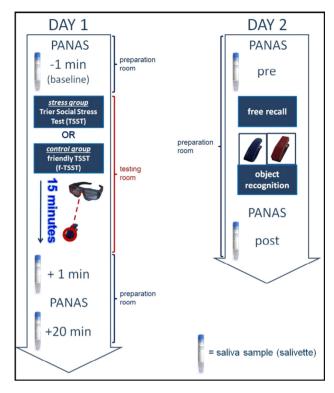


Fig. 1. Experimental time course on day 1 and day 2.

scale, emotional adjectives were rated for their intensity ranging from 1 = 'very slightly or not at all' to 5 = 'extremely'. Answers result in a positive (PA) and a negative affect (NA) score.

2.3.4. Eye tracking recordings

SMI Eve Tracking Glasses 2.0 (SensoMotoric Instruments GmbH. Teltow, Germany) were connected to the appendant notebook for eye tracking recordings which were visible to the committee members on the notebook screen during the procedure. A camera integrated in the eye tracking device records the scene of the participant's field of view, while two eye cameras record the gaze of the participant according to the pupil position assessed by six infrared LEDs on each side. The scene camera has a resolution of 1280×960 pixels with a 60° horizontal and 46° vertical field of view. The gaze tracking range of the eye cameras is 80° horizontally and 60° vertically and their sampling rate was set to 60 Hz. The gaze position accuracy of the device is 0.5° over all distances. The participants are standing at an approximate distance of 1 m. As a consequence, the distance between the eyes of the participant and the objects on the table varies between approximately 1.30 and 1.70 m. Given the accuracy of 0.5°, the error margin is below 1.5 cm. Thus, even the smallest objects used by us (e.g. the rubber) can be tracked with sufficient accuracy. The three images of the different cameras are fused by the eye tracker, such that the participant's fixations are mapped as focus circles onto the scene image. The glasses were adjusted for each participant individually with a selection of nose pads and an adjustable bandeau. Calibration was done by aligning the focus circle as displayed on the output screen of the eye tracking notebook with the actual fixation focus of the participant on three target points, positioned to cover distances and space of the field of view during TSST/f-TSST. The recordings started with the beginning of the preparation phase, five minutes before TSST or f-TSST, respectively, were initiated. When the experimenter re-entered the room, the recordings were stopped. Data were processed with the corresponding SMI software iView 3.5 and analysed with the software BeGaze 3.5.90, both included in the SMI Experiment Center 3.5.

2.3.5. Areas of interest

For data analyses, relevant objects had to be defined to determine the stimuli whereupon participants' fixations would be compared. Our Areas of Interest (AOI), which had manually been marked as such in the software BeGaze, included 10 central and 10 peripheral objects as well as the faces of the committee members. Whereas the central objects were involved in interactions of the committee members, the peripheral objects were static objects at fixed positions on the table (Wiemers et al., 2013). Fixations were determined by a semi-automatic detection mechanism which calculated fixations according to an algorithm of the feature "event detection", provided by the software BeGaze. The assessed events are classified as saccades, visual intakes (>50 ms), and blinks (see the BeGaze manual published by SensoMotoric Instruments, 2016) and include multiple frames making the analyses less time consuming. Two independent raters then assessed whether the fixation shown in the event hit a previously marked AOI, which was the case when the fixation circle overlapped with the AOI. According to the rater's assessment, the software calculated the total fixation time (in ms) for each AOI, representing the mean total fixation duration on the items during the course of the whole trial, and the average fixation time per AOI, which is the average fixation duration across all single fixations on the AOI. This was done separately for central and peripheral objects. As longer fixation duration on an item could be due to staring at it, which would provide no additional information, thus does not necessarily result in a better memory encoding of the object, we also assessed the number of fixations on central and peripheral objects as an additional outcome measure.

Central objects were a beaker, two clipboards, two pencils, a candy box, a rubber, a sharpener, a shelf, a stapler, a timer and a water bottle. Peripheral objects were a book, clips, a mug, a folder, a puncher, a dustbin, a ruler, scissors, a text marker and tissues.

2.3.6. Memory assessment

Participants underwent unexpected free recall and computerised object recognition tasks on the next day. First, participants were asked to orally recall as many of the 20 objects present in the testing room the day before. The answers were compared with a list by the experimenter and checked when having been named. Items were rated as correct when the object was clearly named (e. g. sharpener). Since it rarely occurred that an object was named which was not covered as a central or peripheral object on the list (e. g. table), these replies were not considered for analyses. Further, participants indicated whether they had expected a memory test. The number of items remembered was calculated separately for central and peripheral objects.

The 20 objects which had been in the room the day before, 20 similar objects differing in shape and colour, and 20 unrelated distractor objects were presented in the object recognition task (Wiemers et al., 2013). Participants had to indicate on a 6-point scale how sure they were to have seen the exact object the day before (1 = 'very sure to have seen the object'; 6 = 'very sure to have not seen the object'). Again the mean results for the two item categories were calculated separately.

2.4. Statistical analyses

For all data, mean values were calculated separately for stress and control group and, for the data concerned, separately for central and peripheral objects. If normal distribution was violated, the data were log-transformed. For results from the object recognition task, a discrimination index (DI) was calculated from the raw data to compare memory performance between the groups. First, hit rate and false alarm rate were assessed after a dichotomisation of the replies into the categories "seen", if an object was believed to have been present, regardless of the level of certainty, and "unseen" in the opposite case (Green & Swets, 1966/1974). Hits are participants' correct categorisations of items present during the TSST as remembered, whereas false alarms are items mistakenly categorised as remembered. Hit and false alarm rates are calculated from these variables in relation to the sum of actual and distractor objects. False alarm rate subtracted from hit rate, separately for central and peripheral objects, results in the DIs.

For correlation analyses, differential measures were calculated for cortisol and negative affect (NA). The Area Under the Curve (AUC_i) representing cortisol increase as measured over time, from baseline to 20 min after termination of the stressor, was calculated (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The delta value for the NA was calculated by subtracting the score from the pre-assessment from that of the post-assessment.

The data were analysed using SPSS 22.0.0 for Windows (IBM Corp., New York, USA). They were computed and entered into repeated measures analyses of variance (ANOVA) with between-subjects factors STRESS (stress, control) and SEX (male, female). For the physiological and affective stress measures, we included the within-subjects factor TIME (baseline (-1 min), +1 min, +20 min or pre and post assessment, respectively). Fixation and memory data were analysed including OBJECT TYPE (central, peripheral) as within-subjects factor in the ANOVA.

3. Results

3.1. Participants

Of the 63 participants tested, five did not show up on the second day, for one more participant the calibration of the eye tracking device was not successful, thus the recorded data could not reliably be analysed. Finally, one participant was excluded due to outliers in baseline cortisol (>3 standard deviations (*SD*) from the mean) and one was a cortisol non-responder (delta-cortisol value negative >1.5 *SD*). Of the remaining 55 participants, 29 were in the control and 26 in the stress group.

3.2. Physiological stress measures

3.2.1. Salivary cortisol

As the data lacked normal distribution, the following analyses were conducted with log-transformed data. The assumption of sphericity was violated ($\chi^2(2) = 44.909$, p < 0.001), thus Greenhouse-Geisser corrected *p*-values ($\varepsilon = 0.625$) are reported in the following.

The repeated-measures ANOVA showed a significant TIME - × STRESS interaction (F(1.25,62.5) = 16.563, p < 0.001, $\eta^2 = 0.249$), with an increase of cortisol in the stress and a decrease in the control group (Fig. 2A). Thus, the stress induction was successful. Moreover, a significant TIME × SEX (F(1.25,62.5) = 7.154, p = 0.006, $\eta^2 = 0.125$) interaction was found, with a more pronounced cortisol increase in men, whereas women only showed significant group differences at time point +20.

A post hoc *t*-test for all participants revealed no significant differences between the groups in cortisol level at baseline, but at time points +1 min (t(53) = -4.037, p < 0.001) as well as +20 min (t(53) = -5.133, p < 0.001).

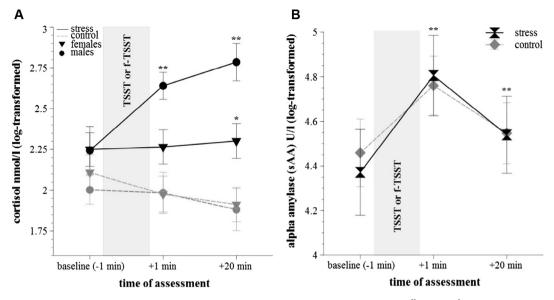


Fig. 2. Mean cortisol (A), and sAA (B) responses for stress and control group at baseline and after the TSST/f-TSST. $p^{*} < 0.001$, $p^{*} < 0.05$ level ((A) both compared to the respective results from the control condition, (B) for changes over time within the groups). Error bars represent standard error of the mean.

3.2.2. Alpha amylase (sAA)

The ANOVA for sAA resulted in a significant effect of TIME (F (2,100) = 24.876, p < 0.001, $\eta^2 = 0.332$), as there was a significant increase in sAA release in both groups 1 min after the TSST or f-TSST, respectively, and decrease 20 min after (Fig. 2B). No other significant effects were found.

3.3. Affect

For the data of the PANAS, separate repeated-measures ANOVAs for NA and PA were conducted.

3.3.1. Negative affect (NA)

For NA, a significant TIME × STRESS interaction, with an increasing NA from pre to post assessment in the stress and a decrease in the control group (Table 1), was revealed (*F*(1,51) = 21.408, *p* < 0.001, η^2 = 0.296). A post hoc *t*-test revealed no differences in NA before (*t*(53) = -0.448, *p* = 0.656), but significantly different NA scores for the post assessment of the NA (*t*(53) = -5.028, *p* < 0.001). Thus, the subjective ratings show an induction of negative affect by the TSST.

3.3.2. Positive affect (PA)

For PA, a significant TIME × STRESS interaction (F(1,51) = 8.778, p = 0.005, $\eta^2 = 0.147$) and a significant within-subjects effect of TIME (F(1,51) = 10.427, p = 0.002, $\eta^2 = 0.170$) were shown, with an increase in the control and no change in the stress group (Table 1). A post hoc *t*-test shows no differences between the groups for the pre assessment of the PA (t(53) = 0.587, p = 0.559), but significant group differences for the post assessment (t(53) = 2.982, p = 0.004). No sex effects for both affect measures were found.

3.4. Memory performance

3.4.1. Free recall

Results from the ANOVA show a significant main effect of STRESS (*F*(1,51) = 48.740, *p* < 0.001, η^2 = 0.489), with superior free recall performance in the stress group. Furthermore, a significant within-subjects effect for OBJECT TYPE (*F*(1,51) = 115.810, *p* < 0.001, η^2 = 0.694), with superior memory for central to memory

Table 1

Mean scores (SD) from the PANAS, separated for negative (NA) and positive affect (PA), before (pre assessment) and after (post assessment) the TSST in the stress and the f-TSST in the control group.

NA	Stress	Control
Pre assessment Post assessment	13.35 (3.21) 16.58 (5.74)**	12.90 (4.12) 10.72 (1.56)*
PA	Stress	Control
Pre assessment	29.50 (5.59)	30.34 (5.08)
Post assessment	29.65 (6.39)	34.52 (5.71)

p = 0.01.

p < 0.001 for differences between pre and post assessment.

for peripheral objects, and a significant OBJECT TYPE × STRESS interaction (F(1,51) = 29.309, p < 0.001, $\eta^2 = 0.365$) were found (Fig. 3A). The stress effect was more pronounced for central than for peripheral objects.

3.4.2. Discrimination index (DI)

The ANOVA for the DI shows a main effect of STRESS (F(1,51) = 6.321, p = 0.015, η^2 = 0.110), with better recognition performance of participants from the stress in comparison to the control group, for central and peripheral objects (Fig. 3B). Moreover, a significant within-subjects effect of OBJECT TYPE could be found, showing a better performance for central compared to peripheral objects for both groups (F(1,51) = 75.041, p < 0.001, η^2 = 0.595).

3.5. Fixation behaviour

3.5.1. Areas of Interest

As two different raters operated the semi-automatic detection mechanism in BeGaze, an intraclass correlation coefficient was calculated for all the assessed measures, fixation duration, average fixation and fixation count (MacLennan, 1993). The correlation coefficient of r = 0.995 demonstrates excellent reliability of the raters' judgement about the fixation parameters.

3.5.1.1. Total fixation duration. The total fixation duration on the AOIs was assessed and the mean fixation duration on central and peripheral objects was calculated separately. The ANOVA showed

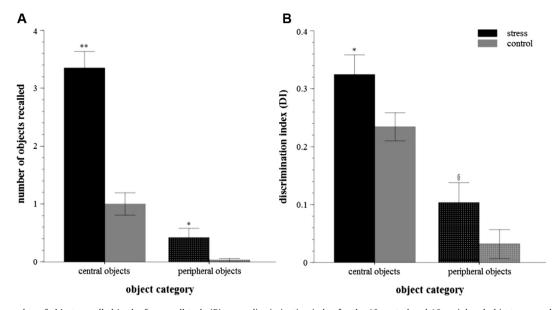


Fig. 3. (A) Mean number of objects recalled in the free recall task, (B) mean discrimination index for the 10 central and 10 peripheral objects remembered in the object recognition task, by participants in the stress and in the control group. * p < 0.001, p < 0.05, compared to the control condition; [§]group difference at a trend level (p = 0.091). Error bars represent standard error of the mean.

a main effect of STRESS with longer fixation times on central and peripheral items by participants from the stress compared to the control group (*F*(1,51) = 15.324, *p* < 0.001, η^2 = 0.231). Furthermore, a significant effect of OBJECT TYPE (*F*(1,51) = 39.603, *p* < 0.001, η^2 = 0.437) and a significant OBJECT TYPE × STRESS interaction (*F*(1,51) = 15.153, *p* < 0.001, η^2 = 0.229) were found (Fig. 4A). Central objects were fixated longer than peripheral objects and the stress effect on the fixation duration was only significant for central objects.

3.5.1.2. Average fixation duration. The average fixation duration for each AOI was calculated for central and peripheral objects and compared between the groups. A main between-subjects effect of STRESS (F(1,49) = 4.194, p = 0.047, $\eta^2 = 0.078$) was revealed by the ANOVA, again with longer mean fixation times of participants in the stress in comparison to those of the control group (Fig. 4B). Moreover, a main within-subjects effect of OBJECT TYPE could be shown, with longer average fixation duration on central in comparison to peripheral objects (F(1,49) = 8.400, p = 0.006, $\eta^2 = 0.146$). A significant OBJECT TYPE × STRESS interaction (F(1,49) = 8.743, p = 0.005, $\eta^2 = 0.151$) showed that this effect was significantly more pronounced in the stress compared to the control group

3.5.1.3. *Fixation count*. Stressed participants fixated the AOIs more often (M = 164.5, SD = 140.12) than control participants (M = 60.4, SD = 62.98). The ANOVA for central and peripheral objects resulted in a main effect of STRESS (F(1,51) = 14.443, p < 0.001, $\eta^2 = 0.221$), with stressed participants exhibiting more fixations on the objects than participants of the control group. Further, a within-subjects effect of OBJECT TYPE (F(1,51) = 53.257, p < 0.001, $\eta^2 = 0.511$) as well as a STRESS × OBJECT TYPE interaction (F(1,51) = 16.308, p < 0.001, $\eta^2 = 0.242$) were shown.

3.5.1.4. Central object fixation. As the objects become central when being manipulated by the committee members, we assessed the fixation behaviour during object manipulation. It shows no significant differences between the groups in fixation of the respective object by the time it was used (F(1,50) = 0.013, p = 0.909). Both groups tended to fixate on average on around 7 of the 10 objects

used by the committee members, [mean (SD)] 6.62 (2.86) in the control and 6.72 (2.48) in the stress group.

Further, we compared fixation count and average fixation times before handling of the objects to these fixation measures from onset of object usage by the committee members¹. Therefore, both fixation measures of the time span before and after the respective object was used were calculated. It was shown that before the respective object was used, stressed and control participants exhibited less frequent fixations (stress: M = 1.86, SD = 2.73; control: M = 1.34, SD = 3.54) than afterwards (M = 7.09, SD = 6.50; M = 3.54, SD = 8.46). Average fixation times were also shorter before (stress: M = 56.25 ms, SD = 55.00 ms; control: M = 36.68 ms, SD = 33.21 ms)than after object use (*M* = 127.75 ms, *SD* = 69.62 ms; *M* = 80.26 ms, $SD = 46.16 \text{ ms})^2$. We conducted a repeated-measures ANOVA with the factors TIME (pre, post) and STRESS (stress, control). For fixation count we found a significant within-subjects effect of TIME (F(1,51)) = 30.681, p < 0.001, $\eta^2 = 0.376$) and a significant STRESS × TIME interaction (F(1,51) = 5.144, p = 0.028, $\eta^2 = 0.092$), demonstrating significantly more fixations from the time of using the objects compared to before, with a pronounced effect in the stress compared to the control group. Similarly, we found a significant within-subjects effect of TIME for average fixation times on the objects (F(1,51))= 61.080, p < 0.001, $\eta^2 = 0.545$) showing significantly enhanced fixation times from the moment the objects became central compared to before. Moreover, a trend towards a STRESS \times TIME interaction (F (1,51) = 3.787, p = 0.057), with a tendency of being more pronounced in the stress group, was found. A main effect of STRESS further reflected generally longer fixation times in the stress compared to the control group (F(1,51) = 7.755, p = 0.007, $\eta^2 = 0.132$).

¹ Note that only 5 of the 10 central objects could be considered for this calculation, as the other 5 were central either from beginning of the trial (stop watch, clipboards, pencils) or only at the very end (stapler, shelf). The remarkable differences to the mean values across all objects described in Section 3.5.1.4 are due to multiple revisits of the clipboards the committee members were taking notes on.

² The mean values reported here include not fixated items (not every participant fixated every single item). When the "0" values for these items are excluded from the calculation, the following mean values are found; <u>stress</u>: M = 141.91 ms, SD = 51.91 ms; <u>control</u>: M = 131.63 ms, SD = 61.63 ms before and <u>stress</u>: M = 167.22 ms, SD = 47.80 ms; <u>control</u>: M = 138.06 ms, SD = 35.81 ms after object use by the committee members.

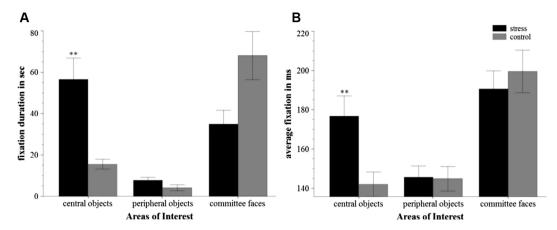


Fig. 4. (A) Mean total, and (B) average central and peripheral object and face fixation times by participants in the stress and in the control group. * p < 0.001 compared to the control condition. Error bars represent the standard error of the mean.

3.5.2. Committee faces

For fixation duration on the committee faces, the ANOVA resulted in a main effect of STRESS (F(1,51) = 4.641, p = 0.036, $\eta^2 = 0.083$), this time with participants from the control group exhibiting longer total fixation times on the committee faces than participants from the stress group, as well as longer average fixation times (see Fig. 4).

3.6. Correlations between the variables

Partial correlations controlling for the factor *stress* showed that fixation duration and number of fixations for central objects were highly correlated (r = 0.934, p < 0.001 for total and r = 0.317, p = 0.020 for average fixation duration), suggesting longer total fixation times to be owed to a more frequent fixation instead of solely staring at the object.

Despite longer average fixation duration as well as an increased memory performance - especially for central objects - in the stress compared to the control group, no correlation between these two measures was found. Moreover, neither cortisol nor negative affect correlated with the two memory measures (Table 2).

To explore the possibility that the longer total fixation times on the objects in stressed participants are related to the gaze avoidance of the committee faces correlations between fixations on faces and objects were calculated. A non-significant positive correlation of total fixation time on objects with total fixation time on the committee faces was shown for the stress group (p = 0.271, r = 0.180) and a non-significant negative correlation between these variables for the control group (p = -0.059, r = 0.760). Across both groups, this resulted in no significant correlation between face and object fixation (p = -0.042, r = 0.760). These results indicate that face and object fixations were not closely related to each other in the entire group as well as in both experimental conditions.

3.7. Mediation analyses

Additionally to the conducted correlation analyses between fixation and memory measures, we performed a formal mediation analysis to investigate whether the influence of stress on memory was mediated by the average fixation duration. The strongest stress effect regarding group differences in memory was found for the free recall performance. Thus, a multiple regression analysis including the parameters stress, average fixation duration and free recall performance was conducted. No full mediation was found (Fig. 5). The explained variation of the predictor fixation on memory had an estimate of $R^2 = 2.5\%$.

4. Discussion

We investigated stress effects on fixation behaviour and its association with memory, 24 h after the stressful experience with a modified version of the TSST featuring office items. Affective and physiological measures demonstrate that the stress induction was successful.

Our memory results show an enhancement of recognition and free recall performance in stressed participants relative to control participants. We observed a pronounced impact of stress on the outcome of the additionally included free recall task. These findings are in line with the results of our previous study applying the object recognition task (Wiemers et al., 2013). In this study, object recognition data showed that differences between stress and control group are based on recollection memory, whereas there were no significant group differences in familiarity. As free recall is based on recollection memory, the results from our study indirectly support the findings from Wiemers and colleagues (2013) and other previous studies on emotional influences on these two memory measures (Anderson, Wais, & Gabrieli, 2006; Kensinger & Corkin, 2003; Sharot, Delgado, & Phelps, 2004; Sharot & Yonelinas, 2008). In an emotional situation, the amygdala responds to arousal whereby binding of items with the emotional component is boosted (Yonelinas & Ritchey, 2015). By this, recollection is supported and the forgetting process is slowed down. These effects, however, do not occur for all stimuli in the same way. Rather, the emotional experience leads to increased memory for central at the expense of memory for the contextual/peripheral aspects of the situation (Mather, 2007). Arousal leads to a bias in perception and encoding towards more relevant stimuli (Mather & Sutherland, 2011). In case of the current study, these are the central objects, as they are linked to the source of emotional arousal which is the committee.

As hypothesised, we could show that participants stressed fixated the objects longer and more often than control participants. We found significant differences for total and average fixation times as well as fixation count. Again, this effect was more apparent for central than for peripheral objects. There were no differences for fixation of the central objects while being handled by the committee members – both groups fixated on average on 7 out of 10 central objects the moment they were used and thus became of relevance. However, from the moment of being handled by the committee members, central objects were being fixated more often and longer than peripheral objects, in particular by stressed participants. This confirms the validity of the categorisation of the objects into central and peripheral objects.

Table 2

Partial correlations of memory data for central objects with physiological (cortisol) AUC_i and affective (NA) stress measures as well as average fixation duration for the central objects (*df* = 52).

Ctrl variable: stress		Cortisol (AUC _i)	NA _{delta}	Average fixation
Free recall	r	-0.108	0.156	-0.076
Central objects	p	0.440	0.264	0.585
DI	r	0.016	0.107	0.107
Central objects	p	0.909	0.444	0.443

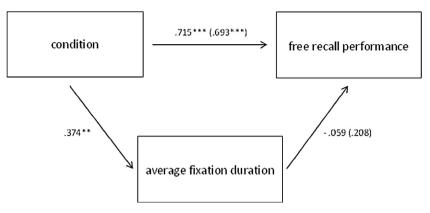


Fig. 5. Mediation analyses with stress being the independent variable, average fixation duration the mediator, and free recall performance the dependent variable. Direct effects are represented by bivariate β -coefficients in parentheses. The standardised β -coefficients from multiple regression analyses are shown as path coefficients. "p < 0.01, ""p < 0.001.

Compared to other studies on natural scene viewing reporting fixation durations of around 250 ms (e. g. Tatler, Gilchrist, & Land, 2005), the average fixation reported by us is comparably low. These differences to other studies are due in part to some participants not fixating all of the objects, which are then included in the calculation as "0" values, lowering the mean. Additionally, differences e. g. in head and eye movement due to the nature of the social interaction might have contributed to the somewhat low average fixation times.

The results of our study suggest that attentional narrowing in a stressful situation and its consequences, as found in previous studies (e. g. Chajut & Algom, 2003), might be related to modified fixation. A study by Christianson, Loftus, Hoffman, and Loftus (1991) also showed more frequent fixations on emotional compared to neutral and unusual but not emotional slides. However, participants' fixation duration in the emotional condition was shorter than in the other two conditions in contrast to our findings. In the current study, the stimuli itself were not emotional or arousing, but their context, which might explain these differences. Similarly to our data, though, there was no direct correlation between number of fixations and memory accuracy. Another study also shows that fixation time is not a predictor for memory performance in terms of hits. Interestingly, though, the number of fixations was a predictor for memory performance in this experiment and fixation duration did co-vary with it (Loftus, 1972). However, work by Posner, using his attentional cuing paradigm, could demonstrate that attention and eye fixation can be dissociated (Posner, 1980). Our results suggest that stress seems to prepare the organism for accurate attentional processing of potentially relevant stimuli by enhancement of the number and duration of fixations.

Faces in every-day life are highly salient and emotional stimuli with a rich informational content (Bahrick & Lickliter, 2014; Caulfield et al., 2016; Crivelli et al., 2016). During a conversation, one naturally focusses on the facial features for emotional feedback. In the control condition (f-TSST), the faces can thus be expected to get fixated often. The affective and stressful component of the TSST added by the committee members, who indeed are the main stressor of the situation, lies in the fact that the participants do not receive any emotional or other kind of feedback by the social agents, turning the committee members into aversive stimuli. As a person's eyes are the most fear-inducing feature in social evaluative situations (Öhman, 1986), gaze avoidance in a social stress situation is a common strategy to reduce discomfort. Voluntary disengagement of attention is a crucial ability for selfregulation (Posner & Rothbart, 2000) and in many different cultures also is an expression of embarrassment and shame (Edelmann & Neto, 1989), most likely elicited in a situation of social evaluative threat. Between the aversion of direct gaze and cortisol a quadratic relation was found in children (de Veld, Riksen-Walraven, & de Weerth, 2014). In a threatening laboratory stress situation like the TSST, adults apparently shift their attention away from the threatening input (Mogg & Bradley, 1998; Mogg et al., 2000; Wilson & MacLeod, 2003). Especially in socially anxious individuals, direct gaze is perceived as a threat, causing its avoidance (Horley, Williams, Gonsalvez, & Gordon, 2003; Wieser, Pauli, Alpers, & Mühlberger, 2009). This explains our results of less facial but increased object fixation times in stressed participants. Since total fixation times on faces and objects are however not correlated and, importantly, do not show a negative correlation in the stress group, the longer fixation times on the objects found in stressed participants appear not to be a direct consequence of their gaze avoidance and decreased fixation of the committee members' faces.

Stress leads to a pronounced bottom-up processing at the expense of top-down control, promoting attentional narrowing towards salient stimuli, whereas less relevant stimuli as well as context are less attended to or even fail to be attended to at all (Arnsten, 2009; Buschman & Miller, 2007; Mather & Sutherland, 2011; Sutherland & Mather, 2012; Sänger et al., 2014). Since we made use of office items which were suited to the situation, the stimuli were not salient by themselves. In contrast to the faces of the committee, which are emotional stimuli, emotional items lack the social component of a face. For future studies, it would thus be interesting to include emotional items (e. g. a knife or a plush toy),

for they are known to produce strong memory effects, in particular in combination with a stressful situation (e. g. Abercrombie, Speck, & Monticelli, 2006; Bradley, Greenwald, Petry, & Lang, 1992; Kuhlmann & Wolf, 2006). Further, when comparing the impact of stress on memory in adults and children (Quas et al., 2014), fixation behaviour might be of additional relevance against the background of the different developmental stages of experience related top-down control and bottom-up influence in adults and children.

As for group differences in fixation times on the objects, partial correlations controlling for condition showed no significant correlations of the stress markers (NA, cortisol, and sAA) or memory measures with fixation data. The subsequently conducted mediation analysis showed average fixation duration to be no mediator between stress and free recall. Thus, even though memory and fixation measures are both influenced by stress, there seems to be no *strong and direct* relation between them. Although the lowest level representational structures of fixated objects are believed to be formed within a few hundred milliseconds and without the requirement of focussed attention (Rensink & Enns, 1998), it is presumed that if focussed attention is not involved, the object representations have very limited coherence in space and time (Rensink, 2000a,b). Thus, object fixation does not warrant the object representation to be consolidated when attention is not focussed towards it. The modified fixation behaviour found in stressed participants might be a prerequisite, but does not guarantee for scene items to be consolidated into long-term memory. It is conceivable that the longer fixation times are driven by the stressful situation to directly manipulate items in visual short-term memory before it is decided whether the items are relevant enough for being consolidated into long-term memory. In future memory studies, experimental manipulation of fixation on stimuli might shed light on the causal relation between fixation behaviour and memory for these stimuli

The stress hormones (nor)adrenaline and cortisol take effect in particular in prefrontal cortex, as well as hypothalamus and hippocampus where a high density of mineralocorticoid and glucocorticoid receptors is located (see for review: Watts, 2005; Wolf, 2009). Together with activation of the basolateral amygdala (see for review: Sara, 2009) and subsequent efferent brain regions (e.g. McGaugh & Roozendaal, 2009), these processes act to differentiate between items worth being transferred into long-term storage and those items (low-level "proto-objects") which may be overwritten by following information (Rensink, 2000a). It has to be emphasised that complex interactions between cortisol, the stress induced negative affect and attentional processing are also, to a considerable extent, responsible for the memory effects under stress (Abercrombie, Wirth, & Hoks, 2012; Wiemers, Sauvage, & Wolf, 2014; Wiemers & Wolf, 2015; Wiemers et al., 2013). Assessing immediate recall performance subsequent to the stress induction, when cortisol peaks, would be an interesting investigation, as this might be more closely correlated with fixation behaviour.

We restricted the group of female participants to women taking oral contraceptives which expectedly led to a blunted free cortisol response (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Nevertheless, stress and control group significantly differed 20 min after the stressor. Furthermore, no other sex differences for the parameters assessed in this study were found and none of our results suggests a relation with the differences in the cortisol response between males and females. The memory effects in females are similar to those found in our former study (Wiemers et al., 2013) and demonstrate that these effects can occur in absence of a strong cortisol response.

In conclusion, our study on the influence of social evaluative threat on fixation behaviour and its possible influence on memory measures shows altered fixation patterns under stress; stressed participants fixate items involved in the stressful situation significantly longer than participants of the control group – an effect which is most prominent for central relative to peripheral objects. At the same time, stressed participants spent less time fixating the faces of the committee members reflecting gaze avoidance. Even though stressed participants also exhibit enhanced memory for central items in the object recognition and free recall tasks, no strong associations of fixation times with memory measures were found. Future studies on scene and object memory of a stressful experience should consider modified fixation and exploration behaviour under stress. Fixation parameters might not stand in direct causal relation to memory outcome, but lead to different preconditions between stress and control group which might mediate the effect of stress on memory at a later stage.

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