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Altered reward learning and hippocampal connectivity following psychosocial stress

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ABSTRACT

Acute stress has a profound influence on learning, as has been demonstrated in verbal learning or fear conditioning. However, its effect on appetitive conditioning is still unclear. Fear conditioning research suggests the possibility of overgeneralization of conditioning to the CS- under acute stress due to its effect on prefrontal and hippocampal processing.

In this study, participants (N = 56 males) were subjected to the *Trier Social Stress Test* or a placebo version. After that, all participants underwent an appetitive conditioning paradigm in the fMRI, in which one neutral cue (CS+) was repeatedly paired with reward, while another (CS-) was not. Importantly, the stress-group revealed overgeneralization of conditioning to the CS- on the behavioral level. On the neural level, stressed participants showed increased connectivity between the hippocampus and amygdala, vACC, and OFC, which maintain specificity of conditioning and also showed reduced differential activation. The results indicate overgeneralization of appetitive conditioning promoted by maladaptive balancing of pattern separation and pattern completion in the hippocampus under acute stress and are discussed with respect to clinical implications.

Introduction

Learning about cues that signal reward is a key element in interactions with our environment. If we repeatedly take a tasty snack out of a blue box, we will soon prefer this blue box over other boxes and our mouth will begin to water as soon as we see it. Previous research showed that this reward learning is altered by acute stress, however, the precise effect of acute stress on reward learning is still unclear (Berker et al., 2016; Lighthall et al., 2013). Reward learning processes can be conceptualized as an appetitive conditioning paradigm, in which a neutral cue (CS+) is repeatedly paired with the chance to win a reward (UCS; e.g. money). Another neutral cue (CS-) is never paired with the UCS. After few pairings, the participants show increased responses to the CS + as compared to the CS- like increased valence and arousal ratings, elevated skin conductance responses (SCRs), and an activation of the reward circuit (Klucken et al., 2015). However, while it is clear that acute stress exerts a profound influence on the reward circuit (Gold et al., 2015; Montoya et al., 2014; Pruessner et al., 2008), its precise effect on appetitive conditioning is still unclear. Studies examining the topic face several difficulties. This includes type and timing of the stressor, as the sympatho-adrenergic response occurs rapidly, while the hypothalamic-pituitary axis (HPA) takes more time to effect the secretion of cortisol (Hermans et al., 2014). Moreover, stress hormones interact with sex hormones and oral contraceptives in females, which often confound effects of gender on emotional learning (Merz et al., 2010; Merz and Wolf, 2017).

The neural circuit underlying reward learning includes the amygdala, the dorsal and ventral striatum, the orbitofrontal cortex (OFC), the anterior insula, as well as the dorsal and ventral anterior cingulate cortex (dACC/vACC) (Haber and Knutson, 2010; Martin-Soelch et al., 2007). Within this circuit, the amygdala is thought to encode the learned CS/UCS-association (Chase et al., 2015). The ventral striatum is assumed to be a key element in the reward circuit, encoding the acquired motivational salience of the cue and CS/UCS contingencies (Klucken et al., 2009). The anterior insula is thought to integrate interoception of emotional reactions with information about the emotional event in reward learning or processing of psychosocial stressors (Kogler et al., 2015; Sescousse et al., 2013). The vACC is thought to play a key role in

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differential conditioning, with a focus on early differentiation between the CS+ and the CS- (Gabriel et al., 2003), while the dACC on the other hand is thought to encode the expected outcome of the CS+ (Alexander and Brown, 2011; Etkin et al., 2011). In addition, the anterior insula has been identified as part of the salience network and is assumed to reflect increased attention towards stimuli associated with reward as well as encoding affective and psychophysiological responses (Chase et al., 2015; Hermans et al., 2014; Kogler et al., 2015).

Previous research on acute stress and conditioning in animals revealed increased generalization of conditioning and impaired goal-directed action under acute stress. In humans, overgeneralization of conditioning from the CS+ to the CS- under stress has previously been discussed in post-traumatic stress disorder (Besnard and Sahay, 2016). In fear conditioning, a network of hippocampus, OFC, and vACC is thought to balance generalization and specificity of conditioning (Xu and Sudhof, 2013). Moreover, this circuit has links to the striatum, amygdala, and midbrain to influence the expression of conditioning. This is in line with research reporting altered functional connectivity of the hippocampus to prefrontal areas as well as the amygdala in fear generalization (Lissek et al., 2014). In this network stress is argued to be an important factor tipping the scales toward generalization of learning (Pedraza et al., 2016). Reduced activation of the vACC, as has also been observed in humans under acute stress (Born et al., 2010; Pruessner et al., 2008), can induce overgeneralization of conditioned responses from the CS+ to the CS- (Cardinal et al., 2003). For the hippocampus, preliminary research suggests that stress impairs pattern separation of different stimuli (Besnard and Sahay, 2016). Structures with ties to the core network mediating specificity of conditioning like amygdala and striatum, which themselves are highly susceptible to stress, are central to the acquisition and expression of conditioning. In a study by Born et al. (2010) participants under acute stress chose more food under stress, while showing reduced activation of amygdala, striatum, hippocampus, and cingulate gyrus toward food cues.

Although the effects of conditioning develop over time, previous research on the effects of acute stress on learning has not taken into account the development of learning in the beginning and in later phases of learning. However, it has been observed that the effects of stress on learning become more pronounced in the late phase. In the dorsal striatum a shift from dorso-medial (caudate) to dorso-lateral (putamen) activation that occurs over time and promotes a shift from goal-directed to habit learning is facilitated under acute stress (Schwabe and Wolf, 2011). It has been suggested that this effect is induced by a deactivation of prefrontal areas, especially the OFC, and a resulting impairment of the executive network (Hermans et al., 2014; Schwabe et al., 2012). In general, under acute stress reduced differential activation of prefrontal and limbic areas has been observed (Dagher et al., 2009; Pruessner et al., 2008).

In the present study, we investigated the altered neural correlates of appetitive conditioning in the fMRI under stress. First, we expected both stressed and non-stressed participants to acquire appetitive conditioning. Second, we expected the stress-group to overgeneralize the acquired conditioning, showing reduced differential neural responses in areas regarding specificity of learning, hippocampus, vACC, OFC, and areas associated with the acquisition and expression of appetitive conditioning, namely the amygdala and the ventral striatum and increased functional connectivity between these structures. In addition, we investigated possible stronger goal-directed activation of the caudate in the control-group during the late phase as compared to the stress-group.

Materials and methods

Participants

A total of 60 male participants (mean age = 23.77 years; SD = 3.03 years) took part in the study. All participants had normal or corrected-to-normal vision and were right-handed, German native speakers with a European background. Exclusion criteria were past or current mental illness, consumption of psychotropic drugs, working in the night shift or travelling across time zones in the past two weeks, and any treatment preventing from entering the magnetic resonance imaging (MRI) scanner. After completion of the experiment, all subjects filled out the German versions of BDI-II (Beck Depression Inventory: Hautzinger, Keller and Kühner, 2009), BIS-15 (Barratt Impulsiveness Scale: Meule, Vögele and Kübler, 2011), and PSS (Perceived Stress Scale: Klein et al., 2016). Prior to the experiment, participants gave written informed consent. After the conclusion of their participation, they received monetary compensation or course credit for their time. Any money they won during the experimental run was paid out directly after participants left the MRI scanner. The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee. Due to technical difficulties data of four participants were excluded from the analysis (3 in the stress-group, 1 in the control-group), leaving 56 participants in the final sample (Table 1).

Procedure

To ensure similar baseline cortisol levels, data acquisition always took place in the afternoon between 1 p.m. and 6 p.m. and participants came into the lab at least 30 min before giving the first saliva sample (Fig. 1). After giving written informed consent, participants performed a training version of the paradigm consisting of different stimuli to familiarize themselves with the task and calculate the speed of their responses in order to adapt the difficulty of the MRI task. Next, participants were prepared for the MRI. Then they gave the first of a total of four saliva samples (Salivette, Sarstedt, Nürnbrecht, Germany), filled out the Positive and Negative Affect Scale (PANAS, Krohne et al., 1996), and were led to a separate room. Here, half the participants (stress-group) took part in the *Trier Social Stress Test* (TSST; Kirschbaum et al., 1993), while the other half (control-group) took part in the placebo version of the TSST (Het et al., 2009). After 20 min, they were led to the MRI, where they gave the second saliva sample and filled out the PANAS a second time. 10 min later they gave a third saliva sample, while in the MRI. The appetitive conditioning paradigm started 10 min after the third sample and a total of 40 min after the beginning of the TSST or Placebo-TSST. This ensured that the appetitive conditioning paradigm would take place after cortisol had been released by the pituitary and reached the brain, which takes about 20 min (Droste et al., 2008). 15 min later the subjects left the MRI, gave the last saliva sample and filled out the PANAS a third time.

TSST/placebo-TSST

The TSST was conducted according to Kirschbaum et al. (1993) in a room with two confederates (one male, one female) in white coats sitting at the head of a conference table. The participants of the stress-group were led in and received written instructions explaining the first task. After 5 min of preparation, one of the confederates instructed the participant to begin. After 5 min, they were given the second task, which again lasted 5 min after which participants could leave the room.

The placebo version was conducted as described by Het et al. (2009) with similar tasks with the same duration as in the TSST, but without elements of uncontrollability or social evaluation. Participants were

Table 1
Descriptive mean (SD) data of stress- and control-group, including age, depression (BDI-II), impulsivity (BIS-15), chronic stress (PSS), sleep duration (hours last night), and total win during the experiment. *p*-values of two-sample-*t*-tests (rightmost column).

	stress-group (n = 27)	control-group (n = 29)	*p*
Age [y]	23.48 (3.30)	23.83 (2.80)	.67
BDI-II	4.59 (4.89)	4.93 (4.50)	.79
BIS-15	33.77 (5.50)	32.51 (5.32)	.40
PSS	13.04 (5.58)	13.19 (4.47)	.92
sleep [h]	7.97 (1.02)	8.05 (0.88)	.76
Win [€]	6.46 (0.13)	6.43 (0.22)	.49

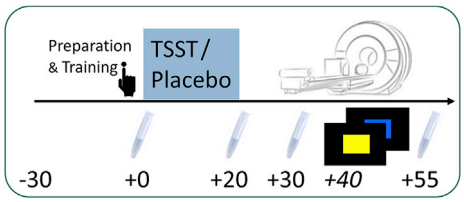

Fig. 1. Schedule of the experiment. Participants arrived about 30 min [-30] before the first saliva sample, and were prepared for scanning and familiarized themselves with the task in the meantime. Directly before the TSST/Placebo-TSST, they gave the first saliva sample [+0], the second sample directly after [+20]. The third sample was given in the MRI before the beginning of MRI measurements [+30], 10 min before the acquisition training [+40]. The fourth and last saliva sample was given after the participants left the MRI [+55].

alone in a room, which the experimenter only entered between tasks to give instructions for the next task.

To assess the stress-inducing effect of the TSST a total of four salivary cortisol samples was collected. Salivette sampling devices (Sarstedt, Nürnbrecht, Germany) were used and then stored at –20 °C until analysis. Samples were assayed using commercially available enzyme immunoassays (IBL International, Hamburg, Germany). Overall cortisol reactivity corrected for baseline cortisol (AUC_i; Pruessner, Kirschbaum, Meinlschmid and Hellhammer, 2003) was then computed. AUC_i differences between groups were analyzed in a two-sample *t*-test using SPSS 22 (SPSS 22.0 for Windows, IBM Inc., Chicago, IL, USA).

Acquisition training

The appetitive conditioning paradigm took place in the MRI scanner and was a modified version of the well-established Monetary Incentive Delay (MID) paradigm (Knutson et al., 2000), adapted as an uninstructed classical conditioning paradigm as previously described in Kruse et al. (2017). A total of 42 trials were presented, 21 CS+ trials and 21 CS- trials. A blue and a yellow rectangle randomly served as CS+ or CS- counterbalanced across groups. 13 of 21 CS+ trials were followed by a win of 0.50€ (reinforcement rate ≈ 62%). The number of trials was designed to exclude the first trial for both CS when learning could not yet have occurred. In addition, the same number of reinforced trials occurred during the early phase (trial 2-11) and the late phase (trial 12-21) to have similar halves for later analysis. Splitting the acquisition in half allows to analyze different phases of the learning process, while minimizing the loss of statistical power (Kolada et al., 2017; Lonsdorf et al., 2017; Tabbert et al., 2005).

Each trial lasted for about 20s with 12s of inter-trial-interval distributed randomly to the beginning and end of the trial (Fig. 2). The CS+ or CS- was then presented for 6s followed by a variable inter-stimulus-interval (1-3s), during which a fixation cross was displayed. This delay was followed by a presentation of the target, a white square, to which the participants had to try to react to by pressing a button while it was visible. While the participants were instructed to always react as fast as possible, a fast enough reaction only led to a win (0.50€) in CS+ trials. To ensure a similar total reinforcement across all participants (aim: 6.50€ ≈ 62% of CS+ -trials) as well as an equal distribution of wins across the whole acquisition phase, the presentation time of the target was varied between 16ms and 750ms. Based on the training session, the mean reaction time (RT_M) and its standard deviation (RT_{SD}) was calculated. The presentation time of the target was longer, when a win was scheduled ($RT_M + 2 \times RT_{SD}$), and shorter, when a loss was scheduled ($RT_M + 2 \times RT_{SD}$). Target presentation times during CS- trials varied accordingly. The target was always directly followed by a presentation of feedback on the win of money and the current balance for 2s.

In the rare case (4.1% of CS+ trials and less than one trial per subject) of an unscheduled win (or loss) because a subject reacted faster (or slower) as established by the training session, a later trial was scheduled to compensate and future response windows were adapted (+20ms for unscheduled losses, -20ms for unscheduled wins).

Subjective ratings

The stimuli serving as CS+ and CS- were rated on the scales arousal, valence and UCS-expectancy before the first training session and the TSST/Placebo-TSST as well as after the acquisition phase. Valence and arousal were rated on a 9-point-Likert scale using the Self Assessment Manikin (SAM, Bradley and Lang, 1994). UCS-expectancy was rated in 10% steps from 0% to 100%. Subjective ratings were analyzed in 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Pre vs Post) analyses of variance (ANOVA) using SPSS 22. Significant interactions were followed up with paired *t*-tests.

Reaction times

During the experiment, button presses in reaction to the target were recorded. Median reaction time data were then analyzed in a 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA using SPSS 22. Significant interactions were followed up by *t*-tests. Moreover, subjects left out targets in 0.06% of trials, with one participant in the control-group not reacting to targets following a CS- throughout the late phase. As group differences with respect to leave-out might skew the analysis of reaction times the number of leave-outs in the respective conditions were analyzed for group differences in a non-parametric Mann-Whitney-U-test using SPSS 22. Post-hoc comparisons were corrected for multiple testing with Bonferroni correction.

Skin conductance measuring

Skin conductance was continuously measured during the acquisition

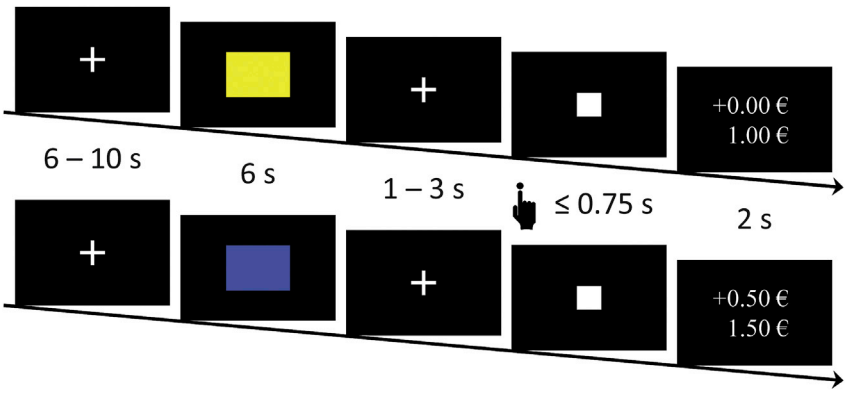

Fig. 2. MID task as in Kruse et al. (2017). Subjects first saw a CS- or a CS+ (colored rectangle). After a variable delay a target appeared for a short time. Subjects were instructed to push a button as soon as the target appeared. In trials that began with a CS+ fast reactions led to a win. After the target vanished, feedback about the win and the current total was displayed.

phase with reusable Ag/AgCl electrodes filled with isotonic (0.05M NaCl) electrolyte medium placed on the non-dominant left hand. Data were collected with a sampling rate of 1 kHz. Ledalab 3.4.4 was used for preprocessing and data analysis (Benedek and Kaernbach, 2010). For preprocessing the data were downsampled to 100 Hz and smoothed with a 32 sample FWHM Gaussian kernel. All data were visually screened. Technical artifacts were spline interpolated. A time window of 1-6s following the onset of CS+ and CS- was defined as analysis window and reflects the CS presentation time. A continuous decomposition analysis (CDA) was performed to separate the underlying tonic response from the specific responding to the stimulus, which was then extracted for analysis. Responding was measured as area under the curve per analysis window after subtraction of the tonic response. All extracted responses were log (μS + 1) transformed to correct for violation of normal distribution of the data. Mean SCRs for CS+ and CS- were calculated for the early and late acquisition phase. Skin conductance data were then analyzed in a 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA using SPSS 22.

fMRI

All MRI images were acquired using a 3 T whole-body tomograph (Siemens Prisma) with a 64-channel head coil. Structural images consisted of 176 T1-weighted sagittal slices (slice thickness 0.9 mm; FoV = 240 mm; TR = 1.58s; TE 2.3s) and were acquired one day later, when participants returned to take part in an appetitive extinction paradigm. This allowed to acquire salivary samples before and after the fMRI sequence as soon as possible. During structural image acquisition a static television test screen was shown. Functional images were acquired with a T2*-weighted gradient echo-planar imaging (EPI) with 36 slices covering the whole brain (voxel size = 3 × 3 × 3.5 mm; gap = 0.5 mm; descending slice acquisition; TR = 2s; TE = 30ms; flip angle = 75; FoV = 192 × 192 mm; matrix size = 64 × 64; GRAPPA = 2). The field of view was positioned automatically relative to the AC-PC line with an orientation of -40°. A total of 432 images were acquired. Statistical Parametrical Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK; 2014) implemented in Matlab 7.14 (Mathworks Inc., Sherbourn, MA) was used for preprocessing the raw data, as well as first and second level analysis. Preprocessing of the EPI images comprised coregistration to the EPI-template provided by SPM, realignment and unwarping (including motion correction), slice time correction, normalization to MNI standard space (resampling to 2 mm isovoxel with trilinear interpolation) as well as smoothing with a 6 mm FWHM Gaussian kernel. Functional data were analyzed for outlying volumes using a distribution free approach for skewed data (Schweckendiek et al., 2013). Each resulting outlying volume was later modeled within the first-level general linear model (GLM) as a regressor of no interest.

On the first level, single subject data were modeled within the GLM with the experimental conditions $CS+_{early}$, $CS+_{late}$, $CS-_{early}$, $CS-_{late}$, UCS+, NoUCS+ and NoUCS-. NoUCS+ and NoUCS- modeled the feedback that no money was won either after presentation of the CS+ or the CS- respectively. The first presentation of the CS+ and the CS- was modeled separately on regressors of no interest as learning could not have taken place yet. CS+ and CS- regressors were split in early (trial 3-22) and late (trial 23-42) phase to assess correlates of early and late learning separately (Davis et al., 2010; Kruse et al., 2017; LaBar et al., 1998; Lonsdorf et al., 2017; Phelps et al., 2004). All regressors were stick functions convolved with the canonical hemodynamic response function. Six movement parameters estimated in the motion correction step of preprocessing were entered as covariates. Additionally, regressors of no interest were entered for the identified outlying volumes, e.g. due to motion during acquisition of a volume which cannot be corrected for by realignment. The time series was filtered with a high-pass-filter (time constant = 128s). For group analysis, the contrasts $CS+_{early} - CS-_{early}$ and $CS+_{late} - CS-_{late}$ were computed.

On the second level, to assess the neural correlates of appetitive conditioning across groups, one-sample *t*-tests were carried out analyzing the neural correlates of acquisition of appetitive conditioning across groups for the contrasts $CS+_{early} - CS-_{early}$ and $CS+_{late} - CS-_{late}$. Secondly, to assess group differences between stress-group and control-group, two-sample *t*-tests were carried out comparing the differential BOLD-response in early and late phase separately ($CS+_{early} - CS-_{early}$ and $CS+_{late} - CS-_{late}$). Cortisol reactivity (AUC_i) was entered as a covariate on separate regressors per group to control for possible interaction effects. Please see the supplemental results for interactions with cortisol reactivity between the groups. Region of interest (ROI) analyses were conducted using small volume correction in SPM12 with $p < .05$ (FWE) and $k > 5$ voxels. Amygdala, anterior cingulate cortex (ACC), nucleus accumbens (NAcc), caudate, orbitofrontal cortex, anterior insula, and hippocampus were selected as regions of interest. If possible, masks were taken from the "Harvard-Oxford cortical and subcortical structural atlases" (probability threshold 0.25) provided by the Harvard Center for Morphometric Analysis. The ACC was separated in the ventral and dorsal part reflecting their differing functions in appetitive conditioning (Etkin et al., 2011; Martin-Soelch et al., 2007). The masks for OFC, ventral, and dorsal ACC were created in MARINA (Walter et al., 2003). In addition, we conducted an exploratory whole-brain analysis for differences between groups, with $p < .05$ (FWE) and $k > 5$ voxels. This analysis yielded no additional results and is therefore not reported.

Functional connectivity (psycho-physiological interaction)

Further, we identified regions changing their functional connectivity in correlation with the acquisition of appetitive conditioning using psycho-physiological interaction analysis as implemented in SPM12 adapting the protocol of Bosch et al. (2017). The chosen seed regions were the bilateral hippocampi. For these regions, the first eigenvariate time series (physiological regressor) were extracted for the whole region of interest and entered into additional first level models alongside the contrast of interest (CS+ - CS-; psychological regressor) and their interaction (psycho-physiological interaction regressor). First level models were otherwise as described above. Separate models were set up for analysis of left and right hippocampal connectivity. On the second level, two-sample *t*-tests were carried out to assess group differences. Cortisol reactivity (AUC_i) was entered as a covariate on separate regressors per group to control for possible interaction effects.

Results

Acute stress reactivity

Salivary cortisol concentrations were analyzed to test the successful induction of acute stress by the TSST (Fig. 3). The experimental groups displayed significantly differing cortisol reactivity in AUC_i ($t(54) = 4.55$; $p < .001$; d = -1.22) with higher values in the stress-group (M = 11.20; SD = 10.41) than the control-group (M = 1.08; SD = 5.74). Critically, the salivary cortisol concentration in the third sample, shortly before the beginning of the experiment, was significantly greater in the stress-group ($t(54) = 5.49$; $p < .001$; d = 1.47).

Subjective ratings

Effects of conditioning were assessed by analyzing subjective ratings of the conditioned stimuli (Table 2). Three 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Pre vs Post) repeated measures analyses of variance (ANOVA) were conducted for ratings of arousal, valence and UCS-expectancy (Table 3); all revealing a main effect of CS as well as a significant CS × Time interaction (all $p < .001$). ANOVA for arousal and UCS-expectancy additionally showed a main effect of Time (both $p < .001$). There was no main effect of Stress exposure (all $p > .32$). Taken together, all rating scales confirm successful acquisition of conditioning.

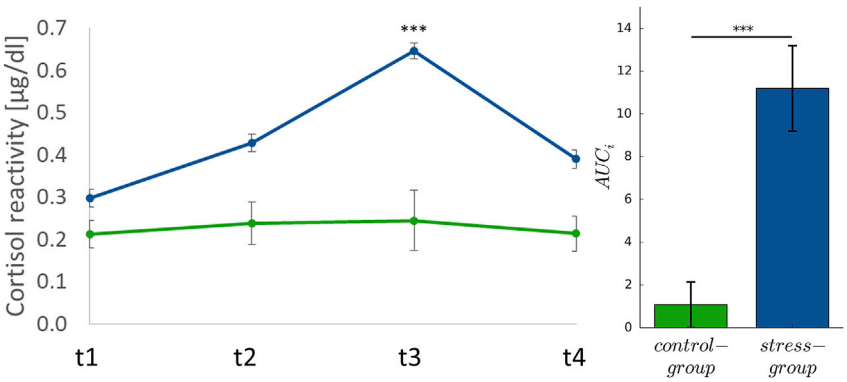

Figure 3. (Left panel) Raw mean salivary cortisol levels at the four sampling points. Significantly increased cortisol level in stress-group at the critical time point t3 shortly before the fMRI paradigm. (right panel) Cortisol reactivity calculated as Area under the Curve corrected for baseline cortisol (AUC_i) plotted for stress- and control-group. Error bars denote standard errors of the mean (SEM). Participants of the stress-group display significantly higher cortisol secretion. *** = $p < .001$.

Table 2
Subjective ratings of the stimuli (CS+ & CS-) with respect to arousal, valence, and UCS-expectancy. Mean (SD) values prior to stress induction and experiment and following the acquisition phase separately for stress- and control-group.

		pre-acquisition		post-acquisition	
		stress-group	control-group	stress-group	control-group
arousal	CS+	3.31 (2.07)	3.21 (1.66)	5.65 (2.31)	5.61 (2.08)
	CS-	3.12 (1.89)	3.57 (1.89)	3.00 (1.77)	3.25 (1.53)
valence	CS+	5.96 (1.46)	5.50 (1.80)	6.77 (1.58)	6.75 (1.71)
	CS-	5.46 (1.79)	5.57 (1.40)	4.42 (1.68)	4.75 (1.69)
UCS-expectancy	CS+	6.27 (1.56)	5.36 (1.87)	7.58 (2.76)	8.18 (1.41)
	CS-	5.92 (1.52)	5.50 (1.84)	0.96 (1.18)	0.82 (0.82)

Table 3
Main effects and interaction effects from 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA for subjective ratings of arousal, valence, and UCS-expectancy with *F*-value, *p*-value, and effect size partial η^2. † = $p < .10$; *** = $p < .001$.

	effect	*F*-value	*p-value*		η^2_{part}
arousal	CS	53.22	<.001	***	.51
	Time	18.79	<.001	***	.27
	Stress exposure	0.17	.685		<.01
	CS × Time	27.13	<.001	***	.34
	CS × Stress exposure	1.62	.208		.03
	Time × Stress exposure	0.03	.873		<.01
	CS × Time × Stress exposure	0.07	.800		<.01
valence	CS	32.01	<.001	***	.38
	Time	0.07	.796		<.01
	Stress exposure	0.002	.968		<.01
	CS × Time	20.18	<.001	***	.28
	CS × Stress exposure	1.18	.282		.02
	Time × Stress exposure	0.75	.391		.01
	CS × Time × Stress exposure	0.07	.797		<.01
UCS-expectancy	CS	219.54	<.001	***	.81
	Time	32.69	<.001	***	.39
	Stress exposure	1.02	.317		.02
	CS × Time	225.23	<.001	***	.81
	CS × Stress exposure	0.07	.793		<.01
	Time × Stress exposure	3.48	.068	†	.06
	CS × Time × Stress exposure	1.80	.186		.03

Reaction times

Investigating reaction times, 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA revealed a main effect of CS ($F(1, 53) = 68.47$; $p < .001$; $\eta^2_{part} = 0.56$) and a main effect of Stress exposure ($F(1, 53) = 5.31$; $p = .025$; $\eta^2_{part} = 0.09$) but not a main effect of Time ($F(1, 53) = 2.74$; $p = .104$; $\eta^2_{part} = 0.50$), which was qualified by a CS × Time interaction ($F(1, 53) = 10.76$; $p = .002$; $\eta^2_{part} = 0.17$) (Fig. 4). As expected, reactions following a CS+ were faster overall and did not improve significantly between the early and late phase ($t(55) = 1.24$; $p = .222$; d = 0.14), while reactions to the CS- slowed from the early to the late phase ($t(54) = -2.74$; $p = .008$; d = -.33). Importantly, there was a CS × Time × Stress exposure interaction ($F(1, 53) = 6.53$; $p = .014$; $\eta^2_{part} = 0.11$). There was no significant Time × Stress exposure ($F(1, 53) = 1.70$; $p = .198$; $\eta^2_{part} = 0.03$) or CS × Stress exposure ($F(1, 53) = 2.14$; $p = .149$; $\eta^2_{part} = 0.04$) interaction. The three-way interaction was driven by a decrease in reaction time in the control-group to targets following the CS- from the early to the late phase ($t(27) = -2.87$; $p = .008$; d = -0.56). The reaction times to targets following a CS- in the stress-group did not differ significantly from early to late phase ($t(26) = -0.64$; $p = .527$; d = -.08). Neither group displayed a

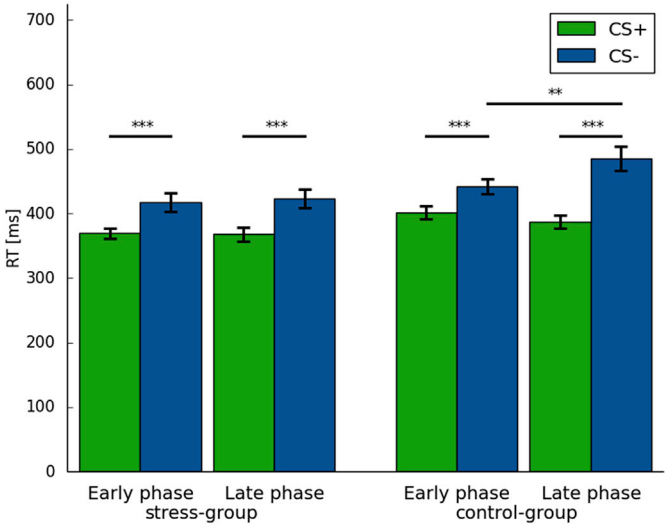

Fig. 4. Mean reaction times [RT] to the targets following the CS+ (green) and the CS- (blue) in milliseconds, separately for stress-group (left) and control-group (right) in early and late phase. Error bars denote standard errors of the mean (SEM). Both stress- and control-group react significantly faster to targets following a CS+. The stress-group shows generally faster reaction times, mainly driven by faster reactions to the CS-. In the late phase, reaction times to targets following a CS- differ significantly between groups, due to slower reactions of the control-group. Reaction times to targets following a CS+ do not differ between groups. $p < .001$ = ***; $p < .01$ = **.

significant change in reaction time to CS+ -targets from early to late phase (stress-group: $t(26) = 0.17$; $p = .866$; d = 0.03; control-group: $t(28) = 1.52$; $p = .140$; d = 0.25). In addition, participants of the control-group also left out reactions to targets more often than the stress-group ($U = 251.5$; $p = .012$; d = 0.60). To test for a CS × Time × Stress exposure interaction in the number of leave-outs as observed in reaction times, Wilcoxon Signed-Ranks tests were performed. In a similar pattern to median reaction times, Leave-outs of a target following the CS- occurred significantly more often in the late as compared to the early phase in the control-group (z = -2.58; $p = .01$; d = -1.09), while there was no such difference in the stress-group (z = -1.10; $p = .272$; d = -.43). There was no difference in leave-outs to targets following the CS+ from early to late phase in either group (stress-group: z = 0.58; $p = .564$; d = 0.22; control-group: z = -1.00; $p = .317$; d = -.38).

Skin conductance responses

On the level of psychophysiological responses, 2 (Stress vs Control) × 2 (CS+ vs CS-) × 2 (Early vs Late) ANOVA was carried out to assess the effect of conditioning in skin conductance responses (SCRs) assessed by continuous decomposition analysis (CDA). There were significant main effects of CS ($F(1, 54) = 37.76$; $p < .001$; $\eta^2_{part} = 0.41$) and Time ($F(1, 54) = 7.47$; $p = .008$; $\eta^2_{part} = 0.12$), indicating higher responses to the CS+ and a general habituation of responses in the late phase. There was no main effect of Stress exposure ($F(1, 54) = 1.56$; $p = .218$; $\eta^2_{part} = 0.03$) and no interaction effects (CS × Stress exposure: $F(1, 54) = 0.21$; $p = .650$; $\eta^2_{part} < 0.01$; CS × Time: $F(1, 54) = 0.002$; $p = .968$; $\eta^2_{part} < 0.01$; Time × Stress exposure: $F(1, 54) = 2.01$; $p = .162$; $\eta^2_{part} = .04$; CS × Time × Stress exposure: $F(1, 54) = 0.36$; $p = .551$; $\eta^2_{part} < 0.01$).

Hemodynamic responses

Main effect of conditioning

Analysis of the pre-defined ROIs across both experimental groups confirmed wide-spread BOLD-responses to the CS+ as compared to the CS- throughout structures of the reward circuit and in both early and late phase (Table 4). Furthermore, we exploratively correlated the differential BOLD responses with the number of leave-outs to both stimuli for both groups separately. While there were no significant correlations in the stress-group, several significant correlations emerged in the control-group. In the early phase, higher differential BOLD to the CS+ as compared to the CS- was associated with increased leave-outs to the CS- in the left ($r = 0.52$, $p = .004$) and right ($r = 0.58$, $p = .001$) amygdala, left dACC ($r = 0.42$, $p = .025$), left hippocampus ($r = 0.40$, $p = .033$), and left OFC ($r = 0.48$, $p = .004$). However, only the right amygdala survives the significance threshold corrected for the number of tests.

Effect of stress exposure (stress vs control)

On the group level, CS+ - CS- were compared separately for the early and the late phase. During both phases, the control-group, as opposed to the stress-group showed overall stronger responses in the contrast CS+ - CS- (Fig. 5). During the early phase, there were stronger differential responses in the right amygdala, the left OFC and the right vACC (Table 5).

In the late phase, higher differential responses were even more pronounced. Significant differences emerged in the left amygdala, left caudate, left hippocampus, bilateral insula left OFC, and the right vACC. Further analyses showed that the stress-group displayed relatively greater activation towards the CS- in the early phase as compared to the control-group for the left OFC ($t(54) = 2.58$; $p = .013$; d = 0.69), and in the late phase for the left caudate ($t(54) = 2.38$; $p = .021$; d = 0.64) and the right vACC ($t(54) = 2.43$; $p = .023$; d = 0.65). Trends were visible in

Table 4
Region of Interest (ROI) results in the contrast CS+ - CS- across groups for early and late phase. Indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected *p*-value (p_{corr}), and effect size (r). † = $p < .10$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

structure	side	k	x	y	z	z_{max}	p_{corr}		*r*
$CS+_{early} - CS-_{early}$									
amygdala	L	171	−24	−2	−12	4.70	<.001	***	.58
	R	92	28	0	−12	3.57	.014	*	.46
caudate	L	493	−16	−6	20	6.83	<.001	***	.73
	R	517	10	10	0	6.51	<.001	***	.74
dACC	L	1334	−6	10	40	6.55	<.001	***	.74
	R	1494	10	12	38	7.14	<.001	***	.78
hippocampus	L	430	−28	−14	−14	4.42	.001	**	.55
	R	500	30	−34	−2	5.14	<.001	***	.62
insula	L	1162	−32	3	14	6.18	<.001	***	.71
	R	1018	34	26	2	6.12	<.001	***	.71
NAcc	L	107	−6	12	−2	5.80	<.001	***	.68
	R	85	10	10	−4	6.24	<.001	***	.71
OFC	L	484	−14	16	−14	4.93	<.001	***	.60
	R	341	14	50	−6	5.53	<.001	***	.66
vACC	L	416	−10	36	18	4.51	<.001	***	.56
	R	666	16	38	22	4.54	<.001	***	.56
$CS+_{late} - CS-_{late}$									
amygdala	L	31	−26	0	−28	3.33	.024	*	.43
	R	25	20	−4	−10	2.93	.077	†	.38
caudate	L	491	−8	4	4	5.75	<.001	***	.67
	R	514	12	12	−2	5.50	<.001	***	.65
dACC	L	1291	−10	12	38	7.24	<.001	***	.79
	R	1381	12	12	42	7.39	<.001	***	.80
hippocampus	L	45	−28	−38	0	3.02	.122		.39
	R	118	34	−30	−6	3.33	.053	†	.43
insula	L	853	−28	24	2	6.58	<.001	***	.74
	R	622	40	12	2	5.88	<.001	***	.68
NAcc	L	107	−14	14	−6	5.71	<.001	***	.67
	R	85	14	16	−6	5.29	<.001	***	.63
OFC	L	205	−14	16	−12	4.79	<.001	***	.59
	R	134	16	16	−12	5.03	<.001	***	.61
vACC	L	35	−12	34	28	3.42	.042	*	.44
	R	131	10	36	32	3.61	.022	*	.46

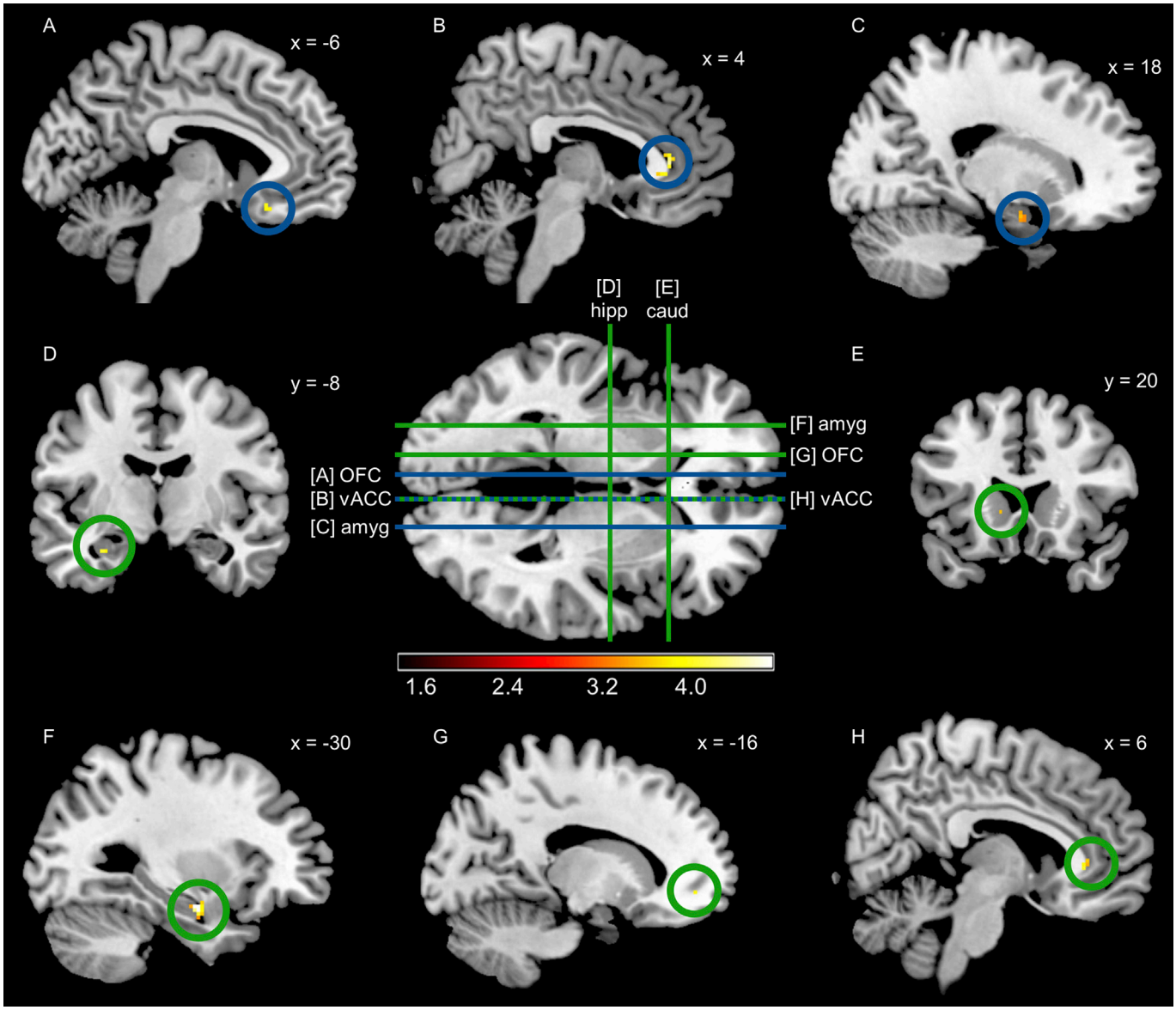

Fig. 5. Significantly increased differential BOLD in the control-group as compared to the stress-group. Upper row [A-C]: results for the early phase include left OFC, right vACC, and right amygdala (amyg) (peak voxels circled in blue). Middle and lower row [D-H]: results for the late phase include left hippocampus (hipp), left caudate (caud), left amygdala, left OFC, and right vACC (peak voxels circled in green). Center image: transversal plane (z = 0) denoting the intersecting planes of the results for the early phase (blue lines) and late phase (green lines). Display threshold is the respective $p < .05$ (FWE corrected) threshold per ROI.

Table 5
Region of Interest (ROI) results for the contrast control-group - stress-group in the contrast CS+ - CS- for early and late phase indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected *p*-value (p_{corr}), and effect size (r). † = $p < .10$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

Control-group – Stress-group										
contrast	structure	side	k	x	y	z	z_{max}	p_{corr}		*r*
CS+ (early) – CS- (early)	amygdala	R	180	18	−2	−18	3.36	.027	*	.44
	OFC	L	341	−6	24	−16	3.62	.037	*	.47
	vACC	R	515	4	36	6	3.98	.007	**	.51
CS+ (late) – CS- (late)	amygdala	L	165	−30	−6	−18	4.28	<.001	***	.54
	caudate	L	379	−12	20	0	3.35	.038	*	.44
	hippocampus	L	242	−30	−8	−22	3.80	.012	*	.49
	insula	L	919	−42	4	−8	4.20	.005	**	.53
		R	81	42	−14	12	4.00	.010	**	.51
	OFC	L	191	−16	48	−6	3.55	.041	*	.46
	vACC	R	239	6	34	0	3.77	.013	*	.48

the early phase for right amygdala ($t(54) = 1.79$; $p = .079$; $d = 0.48$), as well as vACC left ($t(54) = 1.96$; $p = .055$; $d = 0.52$) and right ($t(54) = 1.88$; $p = .066$; $d = 0.50$).

Group differences in functional connectivity

In order to identify possible mechanisms behind the effect of stress psycho-physiological interactions with respect to the contrast of interest (CS+ - CS-) were performed with the left and right hippocampus as seed regions. Analyses revealed stronger connectivity in the stress-group between the left hippocampus and the left amygdala, the right OFC, and the left vACC (Fig. 6). Trends emerged in right amygdala, and the bilateral NAcc and dACC Table 6).

The right hippocampus also showed higher connectivity in the stress-group, with a significant difference in the right dACC, with trends in the bilateral NAcc. These results indicate overall higher hippocampal connectivity in the stress-group.

Table 6
Region of Interest (ROI) results for comparison of stress- and control-group in functional connectivity of the left and right hippocampus for the contrast CS+ - CS-, indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected *p*-value (p_{corr}), and effect size (r). † = *p*<.10; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

structure	side	k	x	y	z	z_{max}	p_{corr}		r
stress-group – control-group (left hippocampus)									
amygdala	L	33	−18	−4	−12	3.21	.045	*	.42
	R	86	20	−4	−10	3.16	.056	†	.41
dACC	L	619	0	−8	50	3.51	.055	†	.45
	R	364	10	6	34	3.42	.077	†	.44
NAcc	L	22	−6	12	−2	2.78	.067	†	.37
	R	17	6	14	−2	2.71	.068	†	.36
OFC	R	501	12	34	−14	3.73	.031	*	.48
vACC	L	301	−8	36	8	4.42	.002	**	.56
stress-group – control-group (right hippocampus)									
dACC	R	71	10	2	34	3.68	.034	*	.47
NAcc	L	12	−6	12	−2	2.88	.051	†	.37
	R	12	6	14	−2	2.65	.077	†	.36

Discussion

The aim of this study was the investigation of the effect of an acute social stressor on appetitive conditioning. Its main focus were altered neural correlates and the identification of possible mechanisms by analyzing functional connectivity. Our analyses first established the success of the experimental stress induction (increased cortisol reactivity in the stress-group) and that acquisition of appetitive conditioning was successful. On the level of subjective ratings both groups rated the CS+ as more positive and arousing and had significantly higher expectancy of reward following the CS+ compared to the CS-. Moreover, in both groups there were increased SCRs to the CS+ as compared to the CS-. Importantly, widespread neural activation to the CS+ as compared to the CS- was visible throughout the reward network, including amygdala, striatum, ACC and OFC, again in the stress-group as well as the control-group.

Effect on the behavioral level

In general, both groups successfully acquired conditioned responses to the CS+. Interestingly, although both groups displayed faster reactions to targets following the CS+, there were group differences in overall responding as well as responding to targets following the CS-. Specifically, the stress-group showed overall faster responses to targets, while both groups were fast enough to win an equal amount of times, which was ensured by the adaptive nature of the paradigm. Most importantly, over time the control-group selectively reduced its effort in responding to targets following the CS-. This was visible in slower reactions and more leave-outs to targets following the CS- during the late phase in the control-group. As the CS- was never paired with a reward, this can be seen as a flexible adaptation of the control-group to the circumstances of the task, which was reduced under acute stress. The results are similar to animal studies of appetitive conditioning under stress. Association of a CS+ with approach behavior led stressed animals to also approach a CS-, that was never paired with a reward (Bussey et al., 1997). In humans, however, previous studies mostly did not include a CS- or had no behavioral element, which the subjects were able to approach without punishment and which was not instructed beforehand as a cue never followed by a reward. In a Go-NoGo-task, participants under acute stress therefore failed to approach a Go-option, while showing no differences to the control-group with respect to inhibiting a response to the punished NoGo-option (Berker et al., 2016). The results are consistent in showing that the stress-group chose to exhibit the same behavior across cues, while the control-group adapted more flexibly. This is in line with theories assuming more generalization on a behavioral level as well as an increase of habitual behavior under acute stress (Pedraza et al., 2016; Schwabe and Wolf, 2011).

From a network perspective, it is assumed that acute stress leads to reduced activation of the executive control network, including prefrontal regions, in favor of increased engagement of the salience network (Hermans et al., 2014). In a Stroop-like task, this pattern of network-activation was observed in subjects showing faster responding

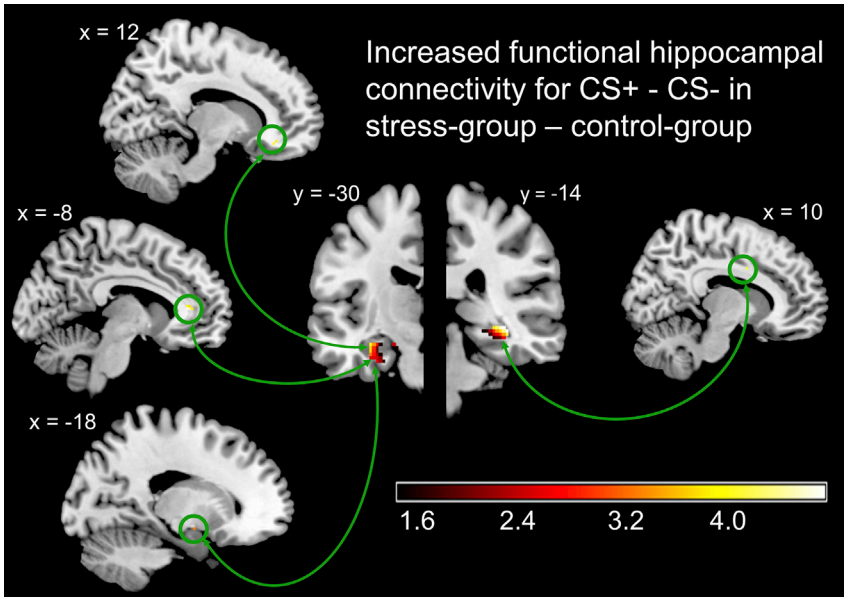

Fig. 6. Significantly increased functional connectivity for the contrast CS+ - CS- in the stress-group as compared to the control-group for the left hippocampus (left side) and the right hippocampus (right side). Functional connectivity of the left hippocampus in the stress-group is increased to right OFC, left vACC, and left amygdala. Functional connectivity of the right hippocampus in the stress-group is increased to right dACC. Display threshold is the respective $p < .05$ (FWE corrected) threshold per ROI.

with reduced accuracy (Kohn et al., 2017).

Effect on neural correlates of appetitive conditioning

On the neural level, greater differential activation in the contrast CS+ - CS- emerged in the control-group in both early and late phase of appetitive conditioning. Namely, there were differences in the amygdala, vACC, and OFC in both phases. Additional analyses showed that the stress-group showed increased responding to the CS- as compared to the control-group in the left OFC in the early phase with trends in right amygdala as well as bilateral vACC, and the left caudate, and right vACC in the late phase. Acute stress increases dopamine release as part of the sympatho-adrengergic stress response in the prefrontal cortex as well as the hippocampus, which presents a possible mechanism in shifting towards overgeneralization of learning (Grace, 2012; Kahnt and Tobler, 2016). Crucially, the group differences were also observed while controlling for cortisol reactivity. This underlines the possibility that it is not the cortisol effect that plays the main role in altered appetitive conditioning but rather early stress effects (e.g. dopamine release). For the associations with stress-induced cortisol-reactivity please see the supplement. Future studies therefore should assess the sympatho-adrenergic response in addition to cortisol reactivity and its effect on dopamine release.

The amygdala has repeatedly been identified to be a key region in classical conditioning (Chase et al., 2011). Within the framework of appetitive conditioning, it seems to encode the association of the CS+ with the UCR (Martin-Soelch et al., 2007). Reduced differential activation under acute stress might indicate impaired differential expression of the conditioned reaction. Viewed by itself, the reduced differential neural activation in the stress-group might also be explained by reduced acquisition of conditioning. Especially in humans, CS- or other additional cues provide context for the perception of the reward indicated by the CS+ (Nieuwenhuis et al., 2005), overgeneralization would possibly alter neural activation towards the CS- as well as the CS+. However, neither in subjective ratings, SCRs, nor reaction times the stress-group showed indicators of reduced acquisition of conditioning to the CS+. Behaviorally, the stress-group only differed in maintained responding to the CS-, a pattern which in animal studies has been identified as overgeneralization. In addition, in the control-group, but not in the stress-group, higher differential BOLD responses to the CS+ as compared to the CS- in the early phase was associated with increased leave-outs to the CS-, underlining the important role of the amygdala in the expression of appetitive conditioning.

In appetitive conditioning, the vACC is thought to be involved in discriminative processes (Gabriel et al., 2003; Parkinson et al., 2000), indicating impaired differential learning under acute stress. Animal findings show that a lack of differential vACC activation in appetitive conditioning leads to continued approach behavior to the CS- in addition to the CS+ (Bussey et al., 1997; Cardinal et al., 2003), which is in line with reduced differential responding as well as reduced differential activation of the vACC in the stress-group.

Under acute stress functioning of the OFC seems to be impaired (Born et al., 2010; Porcelli et al., 2012), resulting in increased habit learning independent of reward value (Schwabe et al., 2012). Besides the reduced differential OFC activation in the stress-group, during the late phase the caudate also showed a reduced differential BOLD response. As a key region in goal directed learning (O'Doherty et al., 2004), this underlines the possibility of reduced goal-oriented learning in the stress-group.

In the late phase, additional group differences emerged in the bilateral insula and the hippocampus with reduced differential activation in the stress-group. In contrast to the early phase, it can be assumed, that subjects have by now formed CS/UCS-associations. A spread of differential activation is in line with more pronounced behavioral differences in the late phase and indicates that the effect of stress does not slow learning but might rather set it on a different path that diverges increasingly.

For a long time, the insula has been thought to mainly play a role in aversive events. However, in line with a recent meta-analysis the insula seems to be involved in emotional processing in general (Sescousse et al., 2013). Moreover, we found reduced differential responding in the stress-group as compared to control-group in the late phase. This might indicate that CS+ and CS- were associated with more similar motivational salience in the stress-group as compared to control-group.

Importantly, a greater differential activation of the hippocampus emerged in the control-group during the late phase. The hippocampus is known as a key region in regulating the stress response via the HPA-axis but is also regarded as an important brain area for learning and memory (Wolf, 2003, 2009). It is thought to serve as a comparator of stimuli, performing pattern separation of CS+ and CS- and pattern completion to adaptively generalize associative learning (McHugh et al., 2007; Treves and Rolls, 1994). In line with the present results, relatively reduced hippocampal activity seems to impair the specificity of learning and foster generalization (Kahnt and Tobler, 2016). Acute stress increases dopamine release in the prefrontal cortex as well as the hippocampus, which presents a possible mechanism in shifting towards overgeneralization of learning (Grace, 2012; Kahnt and Tobler, 2016). One of the few animal studies using a differential conditioning paradigm showed generalization of learning across conditioned stimuli under inhibition of the hippocampus (Yang et al., 2011). But although reduced neural differentiation is in line with overgeneralization of conditioning, it could also be interpreted as reduced learning that is only detected on a neural level, while conditioned reactions are still clearly visible in subjective ratings and SCRs. The neural results do, however, support the assumption of overgeneralization based on the behavioral and translate neural correlates of overgeneralization, that have been identified in animal studies.

Effect on functional connectivity

In addition to significant differences between groups in differential BOLD responses in the hippocampus, functional connectivity analyses with the hippocampus as seed region revealed increased connectivity under acute stress. The pattern of increased hippocampal connectivity includes the amygdala, dACC, vACC, OFC, as well as trend in the NAcc. As several of these areas show reduced differential responding in the stress-group, increased connectivity might point toward inhibitory signaling under acute stress (Giustino and Maren, 2015; Li et al., 2015). Increased functional connectivity between prefrontal and limbic regions has under stress has previously been associated with reduced goal-directed-choice (Maier et al., 2015). Previous studies identified changes in connectivity between the hippocampus and several of these regions as relevant for differential learning and, most importantly, generalization of learning (Dunsmoor and Paz, 2015; Lopresto et al., 2016; Hermann et al., 2016). In animal studies the amygdalo-hippocampal pathway has been implicated in the generalization of fear learning from the CS+ to the CS- (Bergado-Acosta et al., 2008; Sangha et al., 2009). In humans, fear generalization was also associated with increased connectivity between amygdala and hippocampus (Lissek et al., 2014). Regarding prefrontal/hippocampal connectivity, Xu and Sudhof (2013) investigated connectivity in fear generalization. They identified a pathway between the hippocampus and the medial prefrontal cortex in rats (which in humans corresponds to, among others, vACC and OFC), as central for generalization of fear learning. From this central loop, additional pathways influence expression of associative learning via the amygdala, the caudate, and the midbrain.

Future directions and clinical implications

The effect of acute stress on neural networks involved in reward learning has received increasing attention in the recent years with studies reporting reduced as well as increased reward learning (Dillon et al., 2014; Morris and Rottenberg, 2015). While previous research already

highlighted the importance of the timing of the stressor due to changes in the relative involvement of the salience network and the executive control network (Hermans et al., 2014), the present study highlights the possible overgeneralization of learning as a result of reduced prefrontal and hippocampal involvement. Learning took place shortly after the early sympatho-adrenergic stress response. Dopamine, whose release is increased under acute stress, has previously been reported as promoting overgeneralization (Hermans et al., 2014; Kahnt and Tobler, 2016; Lupien et al., 2007). For a better understanding of these effects, however, further studies are necessary that focus on possible molecular mechanisms of different parts of the stress response on these networks to integrate findings on altered network processing with findings on the effect of stress on specific regions.

Generalization of conditioning has long been a focus of clinically motivated research investigating the underlying mechanisms of psychiatric disorders. For example, in addiction, neutral context cues, that have never been directly paired with the consumption of e.g. the drug, lead to craving and induce consumption (Torregrossa et al., 2011). This overgeneralization seems to be related to altered dopamine levels, which have also been a main focus of schizophrenia research (Grace, 2012). Alterations in the reward circuit, possibly induced by stress hormones, have been in the focus of research investigating the development of various disorders such as addiction, depression, or schizophrenia (Bogdan and Pizzagalli, 2006; Grace, 2012; Sinha, 2008). This assumption, however, requires further investigation and transfer of fear generalization paradigms to appetitive conditioning. Moreover, future research should disentangle effects of generalization and habit learning in appetitive conditioning under acute stress.

Limitations

The present study induced acute stress using the well-established TSST. However, in contrast to pharmacological studies investigating the effect of cortisol, the subject cannot be blinded to the condition. Participants were instructed that the TSST was a separate experiment from the learning task, that were both designed to investigate effects of various traits on different tasks. The observed effects may however still be influenced by participants' expectancies about the experiment. Moreover, although the experimenters were not involved in administrating the TSST and did not know the research goals, they were not blinded to the participants' condition. Experimenter bias can therefore not be excluded as a contributing factor to the results. In addition, although men are at a higher risk of developing an addiction, further studies should include women to allow for a generalization of results and investigation of possible sex differences.

Conclusion

In sum, acute stress prior to the acquisition of appetitive conditioning did not impair the acquisition in general. However, it promoted overgeneralization of learning to the CS- on a behavioral level. From a network perspective, an important role comes to increased connectivity under acute stress between the hippocampus and other structures implicated in maintaining to specifity of conditioning, namely vACC and OFC, which show reduced differential activation under acute stress hinting at reduced activation of an executive control network. Moreover, the control-group showed increased differential activation of areas like the caudate, encoding goal-directed action, and amygdala, which was directly associated with reduction of effort towards the CS-. The present study suggests that acute stress promotes the pursuit of reward in the form of generalization to unrewarded stimuli. In the absence of negative consequences it might indeed be adaptive under acute stress to generalize appetitive conditioning instead of optimizing accuracy of learning. The present study suggests generalization of conditioning as a possible mechanism influenced by acute stress that can add to further understand its role in the development of psychiatric disorders.

Conflicts of interest

The authors declared that they had no conflict of interest with respect to their authorship or the publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2017.12.076.

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