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Stress intensifies demands on response selection during action cascading processes

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Summary Stress has been shown to modulate a number of cognitive processes including action control. These functions are important in daily life and are mediated by various cognitive subprocesses. However, it is unknown if stress affects the whole processing cascade, or exerts specific effects on a restricted subset of processes involved in the chaining of actions. We examine the effects of stress on action selection processes in a stop-change paradigm and apply event-related potentials (ERPs) combined with source localization analysis to examine potentially restricted effects of stress on subprocesses mediating action cascading.

The results show that attentional selection processes, as well as processes related to allocation of processing resources were not affected by stress. Stress only seems to affect response selection functions during action cascading and leads to slowing of responses when two actions are executed in succession. These changes are related to the anterior cingulate cortex (ACC). Changes in response selection were predictable on the basis of individual salivary cortisol levels. The results show that stress does not affect the whole processing cascade involved in the cascading of different actions, but seems to exert circumscribed effects on response selection processes which have previously been shown to depend on dopaminergic neural transmission.

1. Introduction

Psychological stress is increasingly recognized as an important modulator of response control processes (e.g. Plessow et al., 2012a,b, 2011; Steinhauser et al., 2007). Stress occurs when the organism senses a disruption or a threat of disruption of homeostasis, leading to a compensatory reaction (Goldstein and McEwen, 2002). Stress is associated with increased catecholamine concentrations and an activation of the hypothalamus pituitary adrenal axis (Arnsten, 2009).

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This is related to feelings of fear, strain, and pressure. Within the field of response control it has been shown that psychological stress leads to impaired task-switching and reduced cognitive flexibility. Even fewer studies examined the role of stress in 'daily situations' where response control on a multitude of different tasks is required, or where different actions have to be chained or cascaded in order to fulfill a task goal (Beste et al., 2013). However, in these situations a multitude of subprocesses involving attentional selection processes, conflict monitoring and response selection contribute to task performance (e.g. Mückschel et al., 2013). Stress may in principle affect all of these processing stages and thereby lead to declines in performance. In other words, it is unclear if stress affects the whole processing cascade, or if the effects of stress are restricted to a subset of processes involved in the chaining of actions.

Using electrophysiological techniques (event-related potentials, ERPs) it is possible to disentangle subprocesses modulated by stress on the basis of distinct ERP components. In the current study we apply ERPs in combination with source localization techniques (sLORETA) to examine which cognitive subprocesses involved in action cascading processes are modulated by psychological stress.

For response selection and conflict monitoring functions the anterior cingulate cortex (ACC) has been shown to be important (e.g. Botvinick et al., 2004; Rushworth et al., 2004). Previous results have shown that stress before most affects response selection processes related to the switching between responses (Steinhauser et al., 2007) when the distance between a cue signaling changes in task structure and a response target is given. This has been interpreted to reflect deficient response selection processes under stress (Steinhauser et al., 2007). From an electrophysiological point of view response selection processes are reflected by the N2 event-related potential, which is also generated by the anterior cingulate cortex (van Veen and Carter, 2002; Folstein and Van Petten, 2008). The N2 has been found to be enlarged when individuals encounter difficulties in response selection or when response selection processes are complicated in case of response switching (e.g. Gajewski et al., 2011, 2012; Beste et al., 2012; Karayanidis et al., 2003; Jackson et al., 2001). As stress has previously been supposed to lead to deficient response selection processes, we expect the N2 to be enlarged under acute psychological stress. To account for the finding that response selection processes are compromised during stress due to impaired response preparation processes we use a stop-change paradigm (SCT paradigm) (cf. Mückschel et al., 2013; Verbruggen et al., 2008) in which we vary the interval between ''stopping'' and ''changing'' and hence the time of the preparation process before the execution of the change response. We expect that stress mostly affects performance (i.e., RT on the change stimuli) in a condition where a preparation of a change response is possible.

However, ''stopping'' and ''changing'' processes are signaled by different sensory modalities: i.e., visual for the stop-stimuli and auditory for the change stimuli. Some results suggest that mechanisms of attentional selection are affected by stress (e.g. Shackman et al., 2011; Elling et al., 2012), but it has to be noted that studies on the effects of stress on attentional selection are inconsistent. It is therefore possible that besides response selection processes also processes earlier in the processing stream (i.e., attentional selection processes) are altered and affect performance. However, we hypothesize that properties of attentional selection processes are only weakly modulated by stress compared to response selection processes for the following reasons: Stress has repeatedly been shown to affect cognitive flexibility (e.g. Plessow et al., 2012a,b, 2011; Steinhauser et al., 2007), which is well-known to be mediated via the dopamine D2 receptor system (e.g. Bertolino et al., 2010; Pezze et al., 2007; Kellendonk et al., 2006). Brain regions modulated by the mesolimbic dopamine (D2) system are well-known to be involved in processes related to response selection and task switching (e.g. Willemssen et al., 2011; Wilson and Bowman, 2005; Botvinick et al., 2004; Rushworth et al., 2004). Opposed to this, attentional selection processes are only indirectly modulated by the dopaminergic system (e.g. Sarter et al., 2006). As attentional selection processes may therefore be not as closely modulated by neurobiological stress responses as response selection processes it is possible that no or only weak effects of stress on attentional selection processes are evident. Visuoperceptual (P1) and attentional selection processes (N1) (e.g. Herrmann and Knight, 2001), or processes related to the allocation of processing resources necessary to relate stimulus processing and responding (i.e., P3) (Mückschel et al., 2013; Polich, 2007; Verleger et al., 2005) and may thus not reveal stress-dependent modulations.

2. Methods

2.1. Participants

In total $N = 30$ male participants were recruited and randomly assigned to the stress group ($N = 15$) and the control group ($N = 15$). Only male participants were enrolled in the study to avoid that fluctuating levels of gonadal steroid hormones induce variance in the data that cannot be explained with the experimental factors used in this study. All participants had normal or corrected-to-normal vision. The participants received course credits or financial compensation for their participation. The study was approved by the Ethics committee of the Ruhr-University of Bochum. Each individual gave written informed consent in addition that experiments were carried out in accordance with the Declaration of Helsinki. Smokers were excluded because nicotine changes the neuroendocrine stress response (Rohleder and Kirschbaum, 2006).

2.2. Paradigm

We used a Stop-Change paradigm, which is shown in Fig. 1. The paradigm is described as in Mückschel et al. (2013), which uses the same paradigm adapted from Verbruggen et al. (2008).

The stimulus display consisted of four vertically arranged circles (8 mm diameter) separated by three horizontal lines serving as reference lines. All stimuli had a vertical viewing angle of 8°. Target stimuli and reference lines were framed by a white rectangle (20 mm × 96 mm). In the first picture of every trial, the potential target stimuli (four empty circles) separated by the three reference lines were presented within

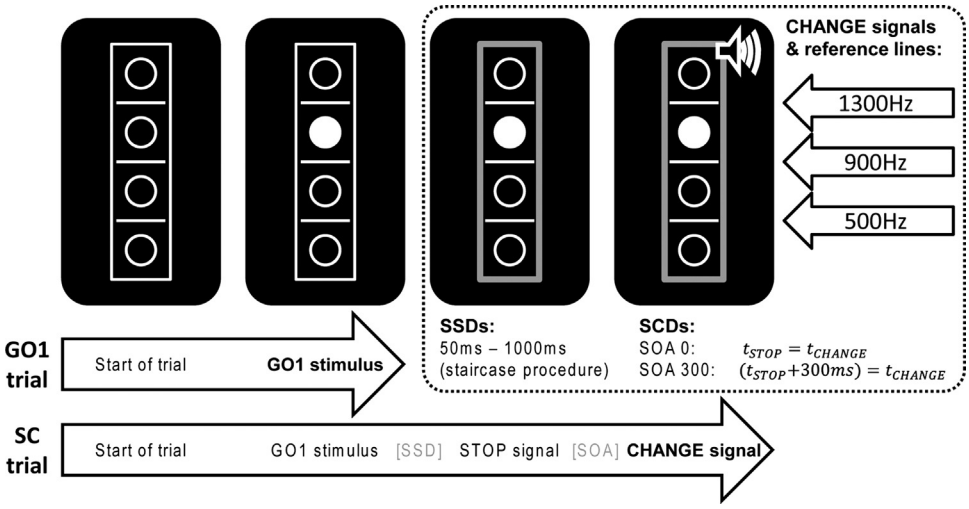

Figure 1 Schematic illustration of the stop-change paradigm. GO1 trials end after the first response to the GO1 stimulus (bold). In contrast, SC trials end after the first response to the CHANGE signal (bold). The stop-signal delay (SSD) between the onset of the GO1 stimulus and the STOP signal was adjusted using a staircase procedure described in Section 2. The stimulus onset asynchrony (SOA) between the onset of the STOP and CHANGE stimuli was set to either 0 or 300 ms. As indicated in the upper right corner, the three CHANGE stimuli were associated with one of the three reference lines.

the white rectangle. After 250 ms, one of the circles was filled with white color (GO1 stimulus). In the GO1 condition, participants were instructed to judge whether this white filled circle (target) was located above or below the middle reference line. In the GO1 condition participants responded by pressing the right outer key with the right middle finger (for ''above'' judgments) or the right inner key using the right index finger (for ''below'' judgments) on a response paddle with four keys. All stimuli remained visible until either the participant responded or a time frame of 2500 ms had elapsed. If no 'stop signal' (a red rectangle replacing the usual white rectangle framing stimuli and reference lines; denoted by gray color in Fig. 1) was presented the GO1 trial ended at this point. The intertrial interval (ITI) was 900 ms. In 30% of all trials, a 'stop signal' was presented. In these cases, a reaction toward the GO1 stimulus had to be inhibited and a new task (''GO2'') had to be executed afterwards, which will be explained below. The 'stop-signal delay' (SSD) was initially set to 450 ms and modified by means of a 'staircase procedure' (see Verbruggen et al., 2008) in order to obtain a 50% probability of successfully interrupted GO1 responses. If a participant fulfilled the requirements of both successfully inhibiting the GO1 response in face of a 'stop signal' and correctly reacting to the subsequent GO2 stimulus, the SSD for the next 'stop/change trial' was prolonged by 50 ms. In case at least one of these two operations failed, the SSD was shortened by 50 ms. The GO2 task was a new judgment following the 'stop-signal' (which persisted until the end of the trial including the presentation of the GO2 stimulus). The GO2 stimulus was presented using two stop-change delays (SCDs) of either 0 ms or 300 ms; i.e., in the SCD0 condition the stop signal and the GO2 stimulus were presented simultaneously, while in the SCD300 condition the stimulus onset asynchrony between stop stimulus and GO2 stimulus was 300 ms. In order to set a new reaction goal for the GO2 part of the trial, a sine tone presented via headphones served as a 'change signal'. There were change signals at three different pitches (low (300 Hz), middle (900 Hz), high (1300 Hz) tone) (presented at 75 dB SPL) indicating which of the three lines replaced the middle reference line if previously set by the GO1 section of the trial. These auditory stimuli were presented via headphones. In case the change signal was a low tone, the low line became the new reference line. Following the same logic, the middle tone encoded the middle reference line while the high tone represented the upper reference line. For the GO2 task, participants responded by pressing the left outer key using the left middle finger (for ''above'' judgments), or the left inner key using the left index finger (for ''below'' judgments). All three reference lines were in effect equally often. The participants were instructed to always respond as fast and accurately as possible. Trials, in which only a GO1 response was required and where stopping and changing to another response was required were randomly intermixed. Moreover, it was not predictable whether the change signal was presented at the same time as the Stop signal, or with 300 ms SOA. Furthermore, the pitch of the tone signaling the change was not predictable. As the pitch of the tone (in relation to the also varying spatial position of the visual stimuli) was also not predictable, it is impossible for the individuals to predict with which finger the alternative response on the change stimuli should be given. All this prevents that preparatory effects in the motor system bias the results. The experiment consisted of a total of 864 trials which were presented within ~40 min (cf. Mückschel et al., 2013).

2.3. Stress induction and cortisol measurement

To induce stress, the socially evaluated cold pressor test (SECPT) was used (Schwabe et al., 2008), as done in previous studies examining action control (Beste et al., 2013). Participants were required to put their left or right foot for 3 min (or until they could no longer tolerate it) into ice water (0—2 °C). Deviating from the usual SECPT protocol we did not use the hand in order to avoid that manual response times (RTs) are

affected. During this phase, participants were videotaped and monitored by an unfamiliar person (social component in the cold water pressure test). Participants in the control condition put their foot into warm water (35—37 °C) for 3 min. They were neither videotaped nor monitored by an unfamiliar person. Participants collected saliva samples before as well as 5, 20, and 50 min after the SECPTor control condition with a Salivette collection device (Sarstedt, Nuembrecht, Germany). Saliva samples were kept at −20 °C until analysis. Free cortisol concentrations were measured using an immunoassay (IBL, Hamburg, Germany). Interassay and intra-assay coefficients of variance were below 10%.

The Stop-Change task was started just after the 5 min saliva sampling point and lasted approx. until the 50 min saliva sampling point. There was a break in the task allowing saliva collection 20 min after the SECPT.

2.4. EEG recording and analysis

Data recording and pre-processing of EEG data is identical to Mückschel et al. (2013) and was done as follows: EEG was recorded from 65 Ag—AgCl electrodes using a QuickAmp amplifier (Brain Products Inc.) at standard scalp positions according to the modified 10/20 system (Pivik et al., 1993) against a reference electrode located at FCz. The sampling rate was 1000 Hz, which was down-sampled off-line to 256 Hz. All electrode impedances were kept below 5 kΩ. Data processing involved a manual inspection of the data to remove technical artifacts. After manual inspection, a band-pass filter ranging from 0.5 to 20 Hz (48 dB/oct) was applied. After filtering, the raw data were inspected a second time. To correct for periodically recurring artifacts (pulse artifacts, horizontal and vertical eye movements and blinks) an independent component analysis (ICA; Infomax algorithm) was applied to the unepoched data set. Afterwards, the EEG data was segmented according to the four different conditions.

Segmentation was applied with respect to the occurrence of the stop-signal (i.e., stimulus-locked). Visual ERPs (due to the stop signal) and auditory ERPs (due to the change signal) were evaluated. Automated artifact rejection procedures were applied after epoching: rejection criteria included a maximum voltage step of more than 60 μV/ms, a maximal value difference of 150 μV in a 250 ms interval or activity below 0.1 μV. Then the data was CSD-transformed (current source density transformation; Perrin et al., 1989) in order to eliminate the reference potential from the data. A second advantage of the CSD-transformation is that it serves as a spatial filter (Nunez and Pilgreen, 1991), which makes it possible to identify electrodes that best reflect activity related to cognitive processes. For a detailed discussion and data on the usefulness of CSD-transformation over other referencing techniques in the stop-change paradigm refer to Mückschel et al. (2013, supplemental material).

After CSD transformation the baseline correction was performed. For the baseline correction we choose a time window from −900 till −700 ms and not a baseline prior to the presentation of the stop stimulus, since we wanted to have a 'real' pre-stimulus baseline that was well before the presentation of the GO1 stimulus. The P1, N1 and P3 ERPs were quantified, based on the scalp topography; i.e., electrodes used for data quantification were selected in a data-driven manner. The peak of the respective components was defined as the maximum negativity (N1, N2) or positivity (P1, P3) within in predefined interval. The intervals were as follows: The visual P1 and N1 were measured at electrode Oz (P1: 0 ms till 140 ms; N1: 150 till 250), the auditory N1 at C3 and C4 (0 ms till 500 ms), the N2 was quantified at electrode Fz (180 till 400 ms) and the P3 at electrode Pz (200 ms till 600 ms). This quantification procedure is identical to the study by Mückschel et al. (2013) examining the principle psychophysiological mechanisms of action cascading using this task. The ERP components were quantified relative to the pre-stimulus baseline. All components were quantified in peak amplitude and latency on the single subject level. The choice of the electrodes was validated as in Mückschel et al. (2013) to ensure that electrodes were used that best picked up activation.

2.5. Source localization (sLORETA)

Source localization was carried out for ERP components that showed modulations in the stressed group, compared to the control group. Source localization was conducted using sLORETA (standardized low resolution brain electromagnetic tomography; Pascual-Marqui, 2002). sLORETA gives a single linear solution to the inverse problem based on extra-cranial measurements without a localization bias (Marco-Pallarés et al., 2005; Pascual-Marqui, 2002; Sekihara et al., 2005). For sLORETA, the intracerebral volume is partitioned in 6239 voxels at 5 mm spatial resolution and the standardized current density at each voxel is calculated in a realistic head model (Fuchs et al., 2002) using the MNI152 template (Mazziotta et al., 2001). In the present study the voxel-based sLORETA-images were compared between groups using the sLORETA-built-in voxel-wise randomization tests with 3000 permutations, based on statistical nonparametric mapping. Voxels with significant differences ($p < .05$, corrected for multiple comparisons) between groups were located in the MNI-brain and Brodman areas (BAs) as well as coordinates in the MNI-brain were determined using the sLORETA software www.unizh.ch/keyinst/NewLORETA/sLORETA/sLORETA.htm.

2.6. Statistical analysis

The behavioral and electrophysiological data was analyzed using mixed effects ANOVAs. In these ANOVAs, "condition (Go, SCD0, SCD300)" served as within-subject factor, "group" (stressed vs. control) served as between-subject factor. For the electrophysiological data an additional within-subject factor "electrode" was introduced wherever necessary. All variables subjected into the ANOVAs were normally distributed as indicated by Kolmogorov—Smirnov tests (all $z < 0.9$; $p > .4$). Greenhouse—Geisser correction was applied and all post hoc tests were Bonferroni-corrected. The p-value cutoff was set at $p = .05$.

3. Results

3.1. Salivary cortisol levels

Salivary cortisol levels were analyzed to examine the neuroendocrinological stress response. The salivary cortisol data

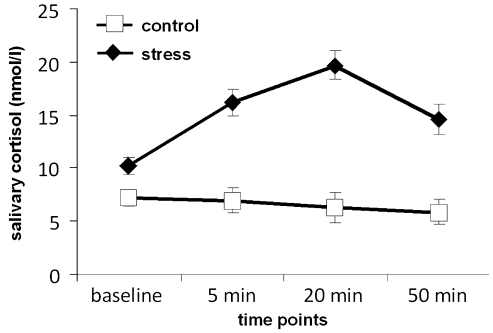

Figure 2 Salivary cortisol level concentrations (nmol/l) the stressed and the control group before, and at various time points after stress induction (5, 20 and 50 min). Means and standard errors of the mean (SEM) are given. Note that the baseline cortisol levels are not different between controls and the stressed group.

was analyzed in a mixed effects ANOVA using time point of salivary probe sampling as within-subject factor and "group" as between-subject factor. Salivary cortisol concentrations across sampling points are shown in Fig. 2.

A main effect "group" showed that salivary cortisol levels were generally higher in the stressed group (15.1 ± 0.9), compared to the control group (6.5 ± 1.1) ($F(1,28) = 45.78$; $p < .001$; $\eta^2 = .913$). There was an interaction "sampling time point × group" ($F(3,84) = 11.72$; $p < .001$; $\eta^2 = .295$) (refer Fig. 2). Subsequent repeated measures ANOVAs within the stressed and the control group showed that salivary cortisol concentrations varied across time points in the stressed group ($F(3,42) = 11.99$; $p < .001$; $\eta^2 = .462$), but not in the control group ($F(3,42) = 1.37$; $p > .2$). In the stressed group, salivary cortisol concentrations were higher 5 min, 20 min and 50 min after SECPT, compared to baseline ($p < .001$). There was no difference in salivary cortisol levels between the groups at the baseline measurement ($p > .4$), but at all other time points ($p < .01$).

3.2. Behavioral data

Behavioral parameters are summarized in Table 1.

Reaction times (RTs) were analyzed in a mixed effects ANOVA using the within-subject factor "condition" (Go, SCD0 and SCD300) and the between-subject factor "group" (stress vs. control). There was a main effect "condition" ($F(2,56) = 11.10$; $p < .001$; $\eta^2 = .284$), showing that RTs were longer in the SCD0 condition (901 ± 38), compared to the SCD300 (740 ± 40) and the Go condition (705 ± 30) (all $p < .003$). The latter conditions (i.e., SCD300 and Go) did not differ from each other ($p > .6$).

Importantly, there was a significant interaction "condition × group" ($F(2,56) = 4.56$; $p = .039$; $\eta^2 = .154$). Independent samples t-tests as post hoc tests revealed no differences in RTs between groups in the GO condition ($t(28) = 1.05$; $p > .2$) and the SCD0 condition ($t(28) = -0.93$; $p > .3$). However, there was a difference between groups in the SCD300 condition ($t(28) = -2.33$; $p = .015$). Here, the stressed group revealed longer RTs ($813 \text{ ms} \pm 41$) than the control group ($668 \text{ ms} \pm 51$).

There were no group effects in the rate of response errors in the SCD0 and SCD300 condition ($F(2,56) = 0.94$; $p > .7$). Analyzing the stop-signal reaction time (SSRT), as calculated after Logan and Cowan (1984), did not reveal differences between the stressed group and the control group ($F(1,28) = 0.45$; $p > .8$). A similar result is observed for the stop-signal delay $F(1,28) = 0.33$; $p > .8$).

Table 1 Behavioral parameters separated for the stressed and the control group (mean ± SEM).

	Stress group	Control group
SSRT	223 ± 12	227 ± 13
SSD	447 ± 40	478 ± 56
RT GO	746 ± 39	735 ± 45
RT SCD 0	910 ± 53	890 ± 51
RT SCD 300	813 ± 41	668 ± 51

3.3. Electrophysiological data

3.3.1. P1 and N1 components

For the electrophysiological data different ERP components were analyzed. At first we examined whether stress modulated attentional selection processes, i.e., attentional processing of the visual "STOP" and auditory "Change stimuli". The visual P1 and N1 are shown in Fig. 3. The auditory and visual P1 and N1 are shown in Fig. 4. For the visual P1 and N1 electrode Oz was analyzed.

For the visual P1 there was no main effect "condition" ($F(1,28) = 0.67$; $p > .7$) and no interaction "SCD interval × group" ($F(1,28) = 2.12$; $p > .3$). For the visual N1 there was a main effect "SCD interval" ($F(1,28) = 6.04$;

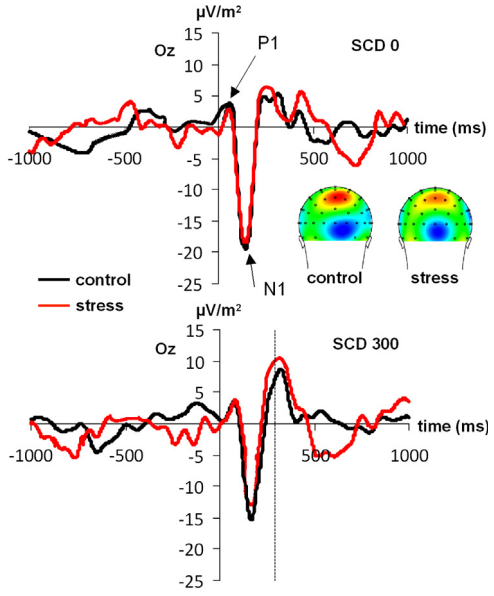

Figure 3 CSD-ERPs traces of the visual P1 and N1 at electrode Oz for the stress (red) and the control group (black). Time point 0 denotes the time point of Stop signal presentation. The plot at the top denotes the SCD0 condition, the bottom plot denotes the SCD300 condition. The topographies denote the visual N1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

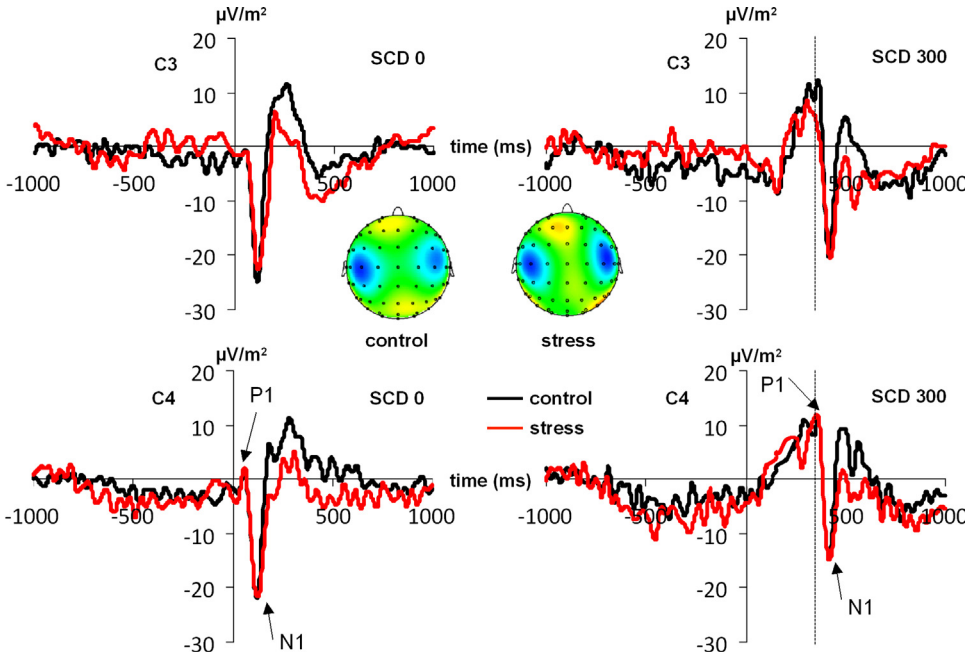

Figure 4 CSD-ERP traces of the auditory P1 and N1 at electrodes C3 (top) and C4 (bottom). The SCD0 condition is shown at the left, the SCD300 condition is shown at the right of the figure. Time point 0 denotes the time point of stop signal presentation. The dashed vertical line denotes the time point of change stimulus presentation in the SCD300 condition. Red lines denote the stressed group, black lines denote the control group. The topographies denote the auditory N1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

$p = .020$; $\eta^2 = .178$) showing that the N1 was larger in the SCD0 (-25.29 ± 3.82), compared to the SCD300 condition (-19.83 ± 3.41). However, there was no interaction "SCD interval × group" ($F(1,28) = 1.11$; $p > .3$). For the P1 and the N1, there was also no main effect "group" (all $F < 0.85$; $p > .7$). There were generally no latency effects for the visual P1 and N1 component (all $F < 1.5$; $p > .2$). These results suggest that stress did not modulate attentional processing of the "STOP stimulus".

As to the attentional processing of the auditory change stimulus, electrode C3 and C4 were analyzed, since these electrodes revealed the center of the negativity in the SCD0 and SCD300 condition. The ERPs of the auditory P1 and N1 are shown in Fig. 4.

A mixed effect ANOVA on the P1 only revealed a main effect "SCD interval" ($F(1,28) = 27.01$; $p < .001$; $\eta^2 = .491$) with the P1 being larger in the SCD300 (3.23 ± 1.30) than in the SCD0 condition (15.25 ± 1.82). However, there was no main effect "group" and no interaction with the factor "group" (all $F < 2.24$; $p > 2$). There was also no main effect "electrode" ($F(1,28) = 0.88$; $p > .3$). A similar pattern of results is observed for the auditory N1. Here, there was also a main effect "SCD interval" ($F(1,28) = 12.15$; $p = .002$; $\eta^2 = .303$) showing that the N1 was larger in the SCD300, compared to the SCD0 condition. However, there was no main effect "electrode", no main effect "group" and no interaction with the factor "group" (all $F < 2.12$; $p > .2$). Besides the necessarily shift in the auditory P1 and N1 latency due to the SCD interval variations, there were no other effects in the latencies (all $F < 0.9$; $p > .3$). These results suggest that stress did not modulate attentional processing of the "Change stimulus".

3.3.2. N2 and P3 components

Previous results suggest that response selection processes and processes mediating between stimulus evaluation and responding critically determine performance in action cascading (Mückschel et al., 2013). To investigate the modulatory effects of stress on these processes we examined the N2 and P3 ERP components and the SCD0 and SCD300 condition.

The N2 was quantified at electrode Fz (e.g. Folstein and Van Petten, 2008). The mixed effects ANOVA revealed a main effect "SCD interval" ($F(1,28) = 7.44$; $p = .004$; $\eta^2 = .237$), showing that the N2 was larger in the SCD0 (-24.22 ± 3.19), compared to the SCD300 condition (-19.24 ± 1.9). There was a main effect "group" ($F(1,28) = 9.28$; $p < .001$; $\eta^2 = .249$) showing that the N2 was larger in the stressed group (-28.89 ± 3.32) than in controls (-14.57 ± 3.45).

There was also an interaction "SCD interval × group" ($F(1,28) = 5.11$; $p = .032$; $\eta^2 = .154$). Post hoc tests revealed that there was no group difference in the SCD0 condition ($t(28) = 1.01$; $p > .2$), but there was a difference in the SCD300 condition ($t(28) = 5.15$; $p < .001$): the stressed group revealed a stronger N2 (-29.07 ± 3.27) than the control group (-9.41 ± 1.95). Paired t-tests within each group showed that the N2 amplitude was not different for the SCD0 and the SCD300 condition in stressed group ($t(14) = 0.11$; $p > .8$). In the control group, however, the N2 was smaller in the SCD300 than in SCD0 condition ($t(14) = -2.98$; $p = .005$). There were generally no latency effects (all $F < 1.1$; $p > .2$).sLORETA analysis was conducted contrasting the stress against the control group in the SCD300 condition. The results suggest that group differences in N2 amplitude are related to differences in activity in the

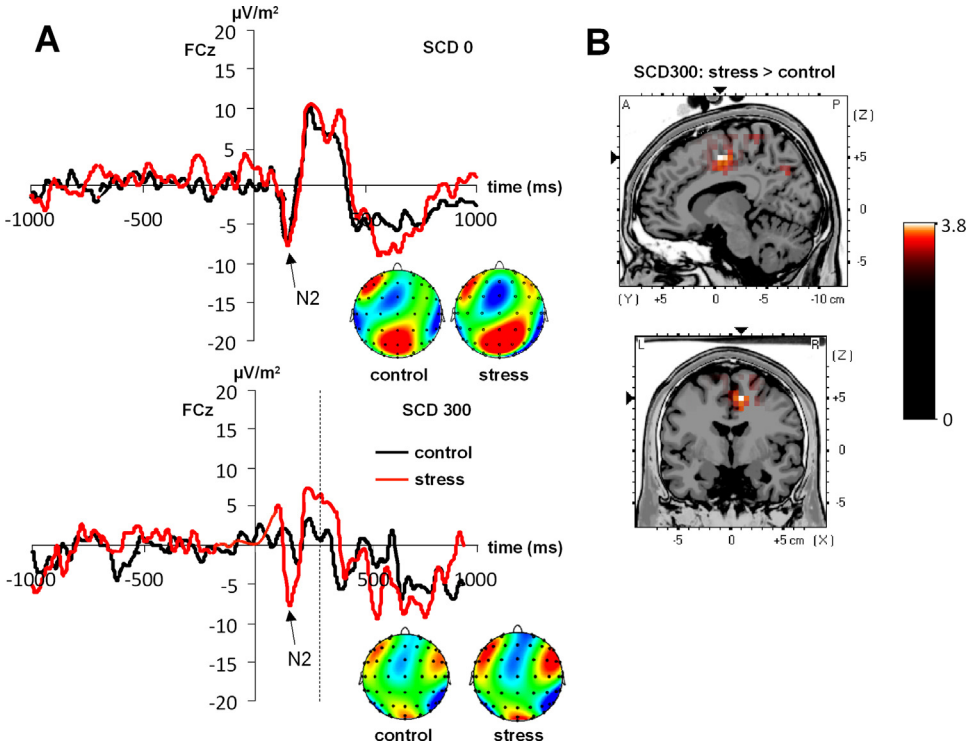

Figure 5 (A) CSD-ERP traces of the fronto-central N2 at electrode Fz for the SCD0 (top) and SCD300 condition (bottom). Time point 0 denotes the time point of Stop signal presentation. The dashed vertical line denotes the time point of change stimulus presentation in the SCD300 condition. Red lines denote the stressed group, black lines denote the control group. (B) Results of the sLORETA analysis showing the source of the group difference in the SCD300 condition within the ACC (BA24). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

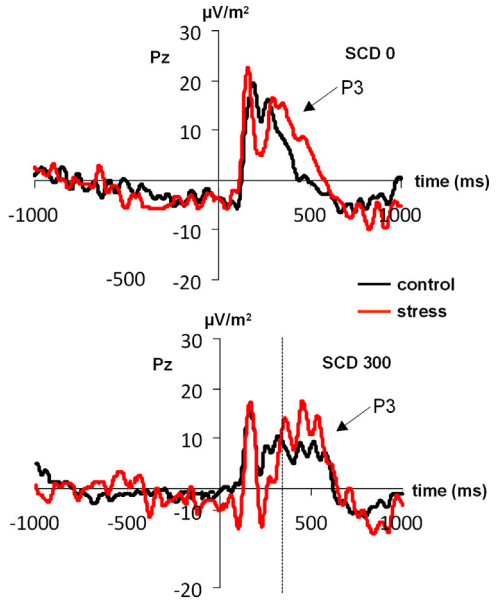

Figure 6 CSD-ERP traces of the P3 at electrode Pz for the SCD0 (top) and SCD300 condition (Bottom). Time point 0 denotes the time point of stop signal presentation. The dashed vertical line denotes the time point of change stimulus presentation in the SCD300 condition. Red lines denote the stressed group, black lines denote the control group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(anterior) cingulate cortex (Fig. 5B) (MNI coordinates: $X = 6$, $Y = 6$, $Z = 36$, BA 24).

Fig. 6 also shows the P3 component at electrode Pz for the SCD0 and SCD300 condition.

As to the P3 component, there was only a main effect "SCD interval" showing that the P3 amplitude was larger in the SCD0, compared to the SCD300 condition ($F(1,28) = 6.35$; $p = .018$; $\eta^2 = .182$). There was no interaction "SCD interval × group" and no main effect "group" ($F < 1.5$; $p > .2$).

3.4. Regression analyses

Regression analyses were carried out to examine whether salivary cortisol concentrations predict alterations in the electrophysiological parameters (ERP components). To this end the mean salivary cortisol concentrations over the 5, 20 and 50 min sampling points were calculated and used in the regression analyses on reaction times and amplitude of the N2 in the SCD300 condition (refer Fig. 7).

For the N2 amplitude a positive correlation is obtained when RTs on the "Change stimulus" in the SCD300 condition are used ($r = .572$; $R^2 = .324$; $p < .001$). These results are shown in Fig. 7 (left). There was a linear inverse correlation showing that the N2 amplitude was larger (i.e., more negative), when salivary cortisol concentrations were higher ($r = -.555$; $R^2 = .302$; $p = .001$) (Fig. 7 right). No such correlation was obtained for the visual and auditory P1 and N1, as well as the P3 in the SOA0 and SOA300 conditions (all $r < .2$; $p > .4$).

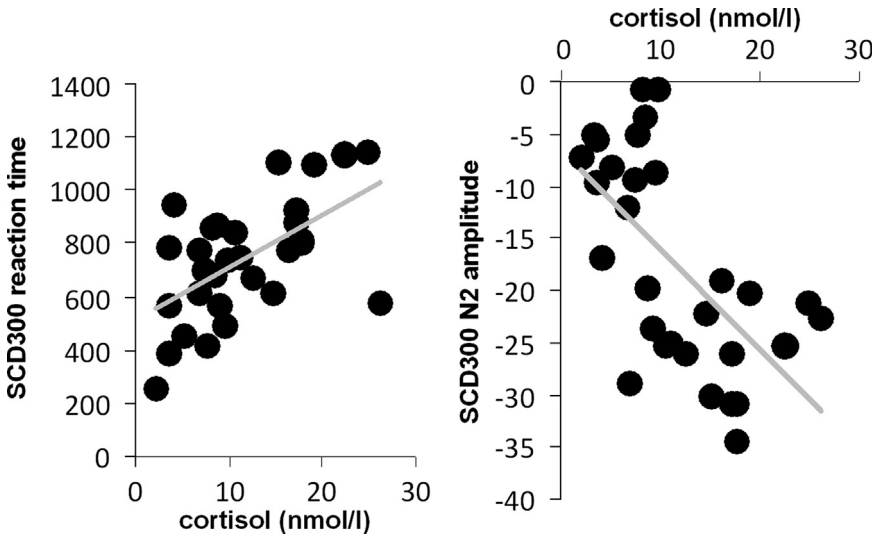

Figure 7 Scatterplots denoting the correlation between salivary cortisol levels and RT in the SCD300 condition (left) and the N2 amplitude in the SCD300 condition (right).

4. Discussion

In the current study we examined the effects of psychological stress on action cascading processes. Goal of this study was to evaluate whether stress specifically affects circumscribed subprocesses involved in action cascading. A limitation of the study is that only male participants were enrolled.

The results show that stress did not modulate action cascading processes when an interruption (stopping) and a change toward an alternative response was required simultaneously. Rather, psychological stress modulated action cascading processes when the change to another response is required when the stopping process has already finished. As revealed by the lack of stress effects on the stop-signal reaction time (SSRT), stress did not modulate the efficiency to stop an ongoing response. This suggests that stress-related processes only affect the change to an alternative response, once an ongoing response has stopped. Previously, Steinhauser et al. (2007) accounted for similar effects in a task-switching paradigm, where they showed that stress predominantly affects performance in a condition with a long cue-target interval. This is similar to the present study where the stop-signal cues the change signal. Steinhauser et al. (2007) suggested that this may be the case because in the high-stress condition task reconfiguration processes do not benefit from anticipation. This may also be possible in the current study.

The electrophysiological data provide insights into the nature of processes that are changed by psychological stress and suggest that stress specifically affects circumscribed cognitive subprocesses related to the response selection stage:

The analyses on the auditory and visual P1 and N1 data revealed no modulatory effect of stress. This is also underlined by the regression analyses showing no linear correlation between cortisol levels and P1/N1 amplitude modulation. Similarly, the P3 was not differentially modulated by stress across SCD conditions and did not correlate with salivary cortisol levels. This shows that visuo-perceptual processes and attentional selection processes (as reflected by the visual and auditory P1 and N1) (e.g. Herrmann and Knight, 2001) do not underlie modulations in action cascading by acute stress. Similarly, the P3 was not modulated in amplitude and latency. Several interpretations have been put forward suggesting that the P3 reflects the allocation of processing resources necessary to relate stimulus processing and responding (P3) (Polich, 2007). Other conceptions suggests that the P3 reflects the response selection bottleneck (e.g. Sigman and Dehaene, 2008; Mückschel et al., 2013; Verleger et al., 2005). As such the lack of modulation in the P3 suggests that these processes remain unaffected by psychological stress in this paradigm.

What seems to be affected by stress, and also corroborates the interpretation by Steinhauser et al. (2007), are response selection processes (see also Oei et al., 2012). The fronto-central N2 showed SCD interval dependent modulations of stress. Besides conflict monitoring (Folstein and Van Petten, 2008; van Veen and Carter, 2002), the fronto-central N2 has also been suggested to reflect response selection functions (Hsieh and Wu, 2011; Gajewski et al., 2010; Swainson et al., 2003) that are modulated in tasks requiring a switch between different actions. The larger N2 in the SCD0 than in the SCD300 condition suggest that in the SCD0 condition the simultaneous requirement of ''stopping'' and ''changing'' induces higher demands on response selection. Stress, however, does not modulate the N2 in the SCD0, but in the SCD300 condition. The sLORETA results suggest that stress-induced activity changes in the SCD300 condition are related to the (anterior) cingulate cortex. Corroborating these results some other fMRI studies also reported that stress affects functioning of the ACC (Stark et al., 2006; Ahs et al., 2006). In the stressed group the N2 in the SCD300 condition was as large as in the SCD0 condition. These results suggests that the demands on response selection are similarly high in the stressed group, despite the processes of ''stopping'' and ''changing'' are not required at once. The time point of change stimulus delivery is highly predictable. However, not predictable is which precise change response is required, since this depends on the quality

of change stimulus upon delivery (i.e., pitch of the tone). It is possible that in stressed participants all possible change responses are represented in prefrontal networks. This likely imposes higher demands on mechanisms selecting between the different response options. This very likely leads to the observed increases in reaction times in the SCD 300 condition in the stressed group. Corroborating this interpretation, the amplitude of the N2 and RTs in the SCD300 condition is predictable by salivary cortisol levels.

One can only speculate about the neurobiological basis of these effects: Previous results suggest that stress affects cognitive flexibility (e.g. Plessow et al., 2012a, 2011; Steinhauser et al., 2007), which is well known to depend on dopamine D2 receptors (e.g. Bertolino et al., 2010; Pezze et al., 2007; Kellendonk et al., 2006). In a strong dopamine D2 state, multiple response representations are evident in prefrontal networks (Durstewitz and Seamans, 2008; Seamans and Yang, 2004), may interfere with each other and increase demands on response selection. In line with this interpretation, the N2 is well-known to be modulated by the dopaminergic system (e.g. Willemssen et al., 2011). However, a putative mechanism related to the dopaminergic system also explains why there were no modulatory effects of stress at the attentional selection stage: This may be due to the fact that sensory cortices are only weakly modulated by dopaminergic projections (e.g. Sarter et al., 2006).

The above interpretation of the N2 effects favor a conception that the N2 reflects response selection processes, which is in line with other studies on tasks where switching between different response programs are relevant (Hsieh and Wu, 2011; Gajewski et al., 2010; Swainson et al., 2003). It is unlikely that the stress-related N2 effects are related to conflict monitoring, since the N2 shows stress-related amplitude increases only in the SCD300 condition, which should be less conflicting than the SCD0 condition, because the two actions are not signaled at once. Furthermore, and as can be seen in Fig. 5b, activation differences are also related to the pre-SMA. This area has frequently been shown to be causally involved in action selection (e.g. Soutschek et al., 2013; Rushworth et al., 2002). The sLORETA results therefore further corroborate in interpretation of stress effects in terms of action selection processes.

In summary, the study examined which cognitive subprocesses involved in action cascading are affected by psychological stress. The results show that stress does not affect the whole processing cascade involved in action cascading, but seems to exert circumscribed effects. Attentional selection processes, as well as processes related to allocation of processing resources were not affected by stress, rather stress seems to specifically affect response selection. These changes are related to the anterior cingulate cortex (ACC) and are predictable on the basis of individual salivary cortisol concentrations. The observed pattern of effects may emerge due to effects of stress on dopaminergic neural transmission.

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The funding source played no role in the design of study, data collection and analyses or discussion of the results, or in the decision to submit and publish the data.

Conflicts of interest

There are no conflicts of interest.

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