The Fatigued Brain: Fatigue and Cognitive Functions in Young Adults

Plukaard, S.C.

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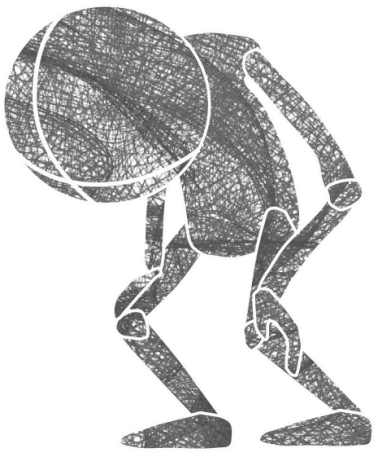
CHAPTER SEVEN

Effects of Prolonged and Acute Fatigue on the Processing and Reappraisal of Negative Emotional Stimuli

Sarah Plukaard, Lydia Krabbendam, Dick J. Veltman, André Aleman & Jelle Jolles

(submitted)



ABSTRACT

Fatigue is a common complaint in modern society and highly prevalent in medical students. As fatigue has been associated with negative mood, increased irritability and reduced cognitive control, we proposed that fatigued students are more responsive to negative stimuli due to weaker, or less effective, cognitive control. The present study used fMRI to compare 26 chronically fatigued medical students to 26 peers without fatigue (mean age: 22 years). Both groups were subjected to a cognitively demanding fatigue manipulation and a non-fatiguing control manipulation to evaluate additional effects of an acute fatigue induction. Brain activation was evaluated during an emotion regulation task that consisted of three conditions: attentively viewing neutral pictures, attentively viewing negative pictures and cognitive reappraisal of negative pictures. The main fMRI findings concerned group differences in response to the viewing of negative pictures: fatigued students showed stronger right amygdala activation, suggesting enhanced emotion reactivity. Moreover, non-fatigued students showed signs of spontaneous reappraisal when viewing negative pictures. This included a functional connection between left amygdala and the dorsolateral prefrontal cortex, which was not present in the group of fatigued students. Effects of the fatigue manipulation suggested reduced visual attention to negative stimuli, and increased prefrontal effort during cognitive reappraisal. With these findings, the present study demonstrated that fatigue in medical students is characterized by differences in the neural dynamics of emotion processing and emotion regulation.

INTRODUCTION

Complaints of fatigue are common in modern society (Bültmann, Kant, Kasl, Beurskens, & van den Brandt, 2002; Ricci, Chee, Lorandeau, & Berger, 2007). Also in the medical students population, which consists of relatively young individuals who are busily engaged in studying, social activities and additional jobs, fatigue is commonly prevalent (Mizuno, Tanaka, Fukuda, Imai-Matsumura, & Watanabe, 2011; Plukaard, Van Batenburg-Eddes, Vos, Croiset, & Jolles, n.d.; Tanaka, Fukuda, Mizuno, Kuratsune, & Watanabe, 2009). The concept of fatigue refers to a perceived lack of energy that interferes with desired activities (e.g., Multiple Sclerosis Council, 1998; Ream & Richardson, 1996). It has a wide variety of causes and underlying conditions such as illness, medication use, stress or unhealthy lifestyle (see (DeLuca, 2005). Moreover, fatigued individuals report difficulties with concentrating and organizing thoughts (e.g., Fisk et al., 1994) as well as altered mood and increased irritability (Dahl, 1999; Leavitt & DeLuca, 2010; Oginska & Pokorski, 2006; Owens, 2001; Ream & Richardson, 1996). However, emotional aspects such as the latter have received little attention in fatigue research. This study aimed to fill this gap by investigating the role of fatigue in emotion processing and emotion regulation.

The relative neglect of emotional aspects in fatigue is remarkable, given the extensively reported co-occurrence of fatigue with depression and anxiety (e.g., Bültmann et al., 2002; Hickie, Koschera, Hadzi-Pavlovic, Bennett, & Lloyd, 1999; Skapinakis, Lewis, & Mavreas, 2003; ter Wolbeek, van Doornen, Kavelaars, & Heijnen, 2006). Fatigue is in itself occasionally referred to as a mood state (Wald & Mellenbergh, 1990) or an emotion (e.g., St Clair Gibson et al., 2003). In university students, fatigue has been negatively associated with emotional intelligence (Brown & Schutte, 2006), which incorporates both the processing and regulation of emotions (Mayer & Salovey, 1995). Building on this research, the present study investigated fatigue in medical students, targeting this link between fatigue and emotion processing.

It has been well established that individuals differ in the strength and nature of emotional

responses (e.g., Canli et al., 2001; Cremers et al., 2010; MacLeod & Hagan, 1992; Robinson, Ode, Moeller, & Goetz, 2007). These differences can depend on the ability to adaptively regulate them (Gross & John, 2003). Emotion regulation is a crucial ability for coping with everyday stressful situations and considered a key aspect of mental health (Gross & Muñoz, 1995). For instance, dysregulation of emotion is connected to higher depressive symptoms (Garnefski & Kraaij, 2006; Silk, Steinberg, & Morris, 2003) and lower wellbeing (Gross & John, 2003; Gross, 2002). Similarly, emotion dysregulation may play a role in the emotional aspects of fatigue.

An effective strategy to regulate emotions is cognitive reappraisal (Goldin, McRae, Ramel, & Gross, 2008; Gross, 1998; Gross & John, 2003). This strategy aims at reducing the emotional impact of a situation by reinterpreting its meaning (Gross, 1999). Neuroimaging studies have found that during cognitive reappraisal, cognitive control regions, including medial and lateral prefrontal cortex (PFC), are responsible for regulation of affective brain regions, such as the amygdala and insula (see Ochsner & Gross, 2005, 2008; Urry et al., 2006). Adaptive emotion regulation by cognitive reappraisal thus relies on adequate cognitive control functions. Remarkably, those are the functions that recurrently show declines in relation to fatigue. For instance, manipulation of fatigue state has resulted in reduced planning ability, cognitive flexibility (van der Linden, Frese, & Meijman, 2003; van der Linden, Frese, & Sonnentag, 2003), attention (Boksem et al., 2005; van der Linden & Eling, 2006), and error monitoring (Boksem, Meijman, & Lorist, 2006; Lorist et al., 2005). Moreover, clinical fatigue is associated with reduced cognitive control as represented by e.g., declines in working memory or attentional control (Cook, O'Connor, Lange, & Steffener, 2007; DeLuca, Genova, Hillary, & Wylie, 2008).

Clinical neuroimaging evidence suggested that associations exist between fatigue and irregular activation of brain areas that are also involved in emotion regulation (Chaudhuri & Behan, 2000; Chaudhuri & Behan, 2004; Cook, O'Connor, Lange, & Steffener, 2007; see Dobryakova, DeLuca, Genova, & Wylie, 2013 for a review). That is, fatigued individuals

show enhanced or more widely distributed activation in PFC and other areas compared to non-fatigued controls. These studies – particularly the ones in which activation increases were observed in the absence of performance declines (Deluca, Genova, Hillary, & Wylie, 2008; Kohl, Wylie, Genova, Hillary, & Deluca, 2009; McAllister et al., 2001) – contributed to the notion that fatigued individuals require more cerebral effort to adequately implement cognitive control.

Given the accumulating evidence for a relationship between fatigue and compromised cognitive control, the emotional aspects of fatigue (e.g., increase in negative mood or increased irritability) may be based on difficulties with cognitive control over undesirable emotions. This notion is in line with other mental health conditions that are characterized by negative emotion and affective imbalance, such as depression or anxiety (Goldin et al., 2009; Rive et al., 2013). During instructed reappraisal, depressed individuals show altered PFC activation (e.g., PFC increases: Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007) and decreased connectivity between amygdala and PFC regions (Heller et al., 2009; Johnstone et al., 2007). Altered PFC recruitment or irregular PFC functional connectivity may similarly characterize fatigued individuals. On the other hand, it is plausible that fatigue involves increased reactivity to affective stimuli, which corresponds to recent observations in fatigue-related circumstances such as sleep deprivation (S. Yoo, Gujar, Hu, Jolesz, & Walker, 2007). Sleep deprived healthy individuals showed enhanced amygdala activation compared to a control group (not sleep deprived) in response to aversive images. Such increases in emotion reactivity are thought to result from reduced prefrontal regulation (Campbell-Sills et al., 2011; Gujar, Yoo, Hu, & Walker, 2009; M L Phillips, Ladouceur, & Drevets, 2008; S. Yoo et al., 2007). Likewise, individuals with prolonged fatigue may be characterized by enhanced amygdala response to negative emotional situations, due to a reduction in spontaneous top-down control.

Current study

The goal of the present study was to investigate whether and to what extent fatigue in medical students involves changes in the processing and regulation of negative emotion.

We focused on medical students since this population appears to be vulnerable to fatigue. Furthermore, medical students are relatively homogeneous in terms of intelligence and educational background. We deliberately limited our sample to females, since males and females differ substantially in their emotional response to affective stimuli (Bradley, Codispoti, Sabatinelli, & Lang, 2001; Fischer, 2000) as well as the cognitive regulation of emotion (McRae, Ochsner, Mauss, Gabrieli, & Gross, 2008).

Fatigue in this study was operationalized in two ways; first, we investigated differences between medical students with high fatigue of prolonged duration (i.e., longer than two months, from here on referred to as 'chronic fatigue') and students without fatigue. Second, we studied the effect of an acute fatigue manipulation. To this end, we experimentally manipulated cognitive fatigue in both groups by a fatigue inducing manipulation (i.e., 90 minutes of performance on cognitively challenging tasks) in one session and a control manipulation (i.e., 90 minutes of non-challenging activity) in another session. The rationale behind this double approach is that acute fatigue and chronic fatigue may act upon common cognitive mechanisms, such as changes in neural effort (see for example Boksem & Tops, 2008 and Dobryakova, DeLuca, Genova, & Wylie, 2013). Moreover, prior research has indicated that group differences in neural activation can depend on such a manipulation (Klaassen et al., 2014).

As fatigue-related differences are not always detectable at the behavioral level (e.g., Lange et al., 2005), we used functional magnetic resonance imaging (fMRI). In addition, the pattern of neural activation could clarify whether potential differences comprise emotion reactivity (e.g., changes in amygdala response) or cognitive regulation (e.g., changes in cognitive control regions). We used an emotion regulation task that previously elicited robust reappraisal-related activation in PFC areas as well as down-regulation of affective areas (Ochsner, Bunge, Gross, & Gabrieli, 2002). Moreover, this task proved to be sensitive to differences between healthy young individuals with varying trait characteristics (e.g., psychotic proneness: Modinos, Ormel, & Aleman, 2010[a] and dispositional mindfulness: Modinos, Ormel, & Aleman, 2010[b]). We anticipated that

fatigued students would process negative emotional stimuli differently compared to their non-fatigued peers due to less effective or less efficient top-down cognitive control. More specifically, we hypothesized that fatigued students would show enhanced amygdala response to negative stimuli and altered recruitment of prefrontal brain areas during reappraisal. In line with clinical evidence, fatigued students possibly exert more cerebral effort in order to adequately regulate their emotions. Second, if such differences are present, we expected decreased functional connections between amygdala and the PFC. When viewing negative stimuli, this would suggest decreased automatic regulation, whereas in case of instructed reappraisal it would more likely refer to the explicit ability to regulate emotions. Third, the extent to which individuals spontaneously engage in emotion regulation (i.e., in the absence of instructed reappraisal) can depend on differences in habitual use of reappraisal strategies in everyday life (Drabant, McRae, Manuck, Hariri, & Gross, 2009). Examination of spontaneous emotion regulation is ecologically most relevant, since individuals generally do not receive explicit instructions from others to regulate their emotions. Therefore, we additionally investigated group differences in the context of habitual use of cognitive reappraisal to regulate emotions.

METHODS

Participants

Twenty-six fatigued females (age range 19.0 – 30.6 years, *M* = 21.8, *SD* = 2.3) and twenty-six non-fatigued females (age range 18.8 – 28.2 years, *M* = 21.6, *SD* = 1.9) were included in the analyses. All participants were right-handed and had normal or corrected-to-normal vision and no hearing problems. All reported no history of medical, neurological or psychiatric disorder, and no use of medication (apart from contraceptive drugs).

Participants were recruited via a survey distributed among all first, second and third year medical students (i.e., the pre-clinical stage of medical school) at the VU University Medical Center Amsterdam. Out of 1050 eligible students, 701 students returned the completed questionnaire (67% response rate). We selected students who reported fatigue complaints for longer than 2 months and who scored above 76 on the Checklist

Individual Strength (CIS; Vercoulen et al., 1994) for the *fatigue group*. The cutoff point of >76 was determined by Bültmann and colleagues (Bültmann et al., 2000), and indicates a fatigue level at which individuals are at risk of subsequent sickness absence. Of the 701 survey respondents, 31% scored above this threshold. For the *control group*, we selected students who reported no fatigue complaints and who scored below 65 on the CIS (i.e., the mean score of all respondents; SD = 21). Although the students were recruited during the pre-clinical stage of medical school, five participants from the fatigue group and four from the control group started their clinical internships by the time they participated in the study.

All participants gave written informed consent prior participation and received financial compensation. This study was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam.

Procedure

The participants completed a 1.5 h. training session and two 3 h. test sessions on three separate days. The training session was scheduled within a week before the first test session. During the training session, participants completed the emotion regulation questionnaire (ERQ; designed to assess habitual use of cognitive reappraisal and expressive suppression to regulate emotions; Gross and John, 2003), a self-report depression scale (CES-D Scale; Radloff, 1977), the CIS (once more) and several neuropsychological tests that served to compare the groups on basic cognitive abilities. The tests included the 20-minute version of the Raven Advanced Progressive Matrices Test (RAPM; Raven et al., 1993; Hamel & Schmitmann, 2006) and the fourth version of the Peabody Picture Vocabulary Test (PPVT-IV; Dunn & Dunn, 2005) as measures of non-verbal and verbal intelligence, the Letter Digit Substitution Task (LDST; Van der Elst, Van Boxtel, Van Breukelen, & Jolles, 2006[a]) as a measure of information processing speed, the Concept Shifting Task (CST; Van der Elst, Van Boxtel, Van Breukelen, & Jolles, 2006[b]) and the Stroop Color-Word Test (Stroop, 1935) as measures of cognitive flexibility. The training session ended with a practice version of the fMRI tasks.

The test sessions took place on weekend days with one or two weeks apart, and started at 14:00 or at 15:30 h. The test sessions commenced outside the scanner with a fatigue manipulation in one session (i.e., fatigue session) and a control manipulation in the other session (i.e., control session; the order of the sessions was randomized). Apart from the manipulation, both test sessions were identical. The fatigue manipulation consisted of 20 minutes of mental arithmetical calculations, followed by 20 minutes of brainteaser puzzles (such as arithmetic sequences and syllogisms), 20 minutes of a computerized Stroop task (Stroop, 1935) with extra auditory interference, adopted from Evers and colleagues (Evers, Van der Veen, Jolles, Deutz & Schmitt, 2009) and an N-back computer task (2- and 3-back) for 30 minutes (see also Klaassen et al., 2013 for details). During the control manipulation, the participants spent 1.5 h. reading magazines or watching documentaries (a collection of magazines and documentary style DVD's was provided). Subsequently, the participants were as quickly as possible transferred to the scanner in which they performed the emotion regulation task and two other tasks that are described elsewhere.

Questionnaires

The CIS consists of 20 statements that measure different aspects of fatigue: fatigue severity (8 items, e.g., "I feel tired"); concentration (5 items, e.g., "my thoughts easily wander off"); motivation (4 items, e.g., "I'm looking forward to many fun things to do"); and physical activity (3 items, e.g., "I don't do much during the day"). Respondents rated how they felt during the previous two weeks. Responses were scored on a 7-point Likert scale, ranging from 1 "Yes, that is true", to 7 "No, that is not true". The sum of all scales yields a fatigue score ranging from 20 to 140. An additional question required the participants to indicate whether they suffered from fatigue complaints for longer than two weeks. If they answered this question with yes, they were asked to specify how long they experienced fatigue by choosing one of the following options: "from two weeks to one month", "from one to two months", "from two to three months", "from three to four months" or "longer than four months".

The ERQ consists of 10 statements that measure individual differences in the everyday use of emotion regulation strategies. It contains two subscales: cognitive reappraisal (6 items, e.g., "I control my emotions by changing the way I think about the situation I'm in") and suppression (4 items, e.g., "I control my emotions by not expressing them"). Responses were scored on a 7-point Likert scale, ranging from 1 "strongly disagree", to 7 "strongly agree". The scores for each scale were based on the mean and ranged from 1 to 7.

The CES-D consists of 20 questions related to feelings of mental depression (16 items, e.g., "I felt depressed") or to the absence of depression (4 items, e.g., "I was happy") during the last week. Four answers were possible at each question: seldom or never (< one day), sometimes (1-2 days), regularly (3-4 days), and often or always (5-7 days). Total CES-D scores ranged from 0 to 60.

The fatigue Rating Scale Mental Effort (RSME; Zijlstra, 1993) was administered to assess the effect of the manipulation on subjective levels of fatigue and mental effort. The scale was completed before (T0) and after the manipulation (T1), as well as after scanning (T2). The RSME contains seven visual analog scales (range: 0 – 150) that measure different aspects of fatigue, including required effort for focusing of attention, tiredness and boredom (e.g., "How much effort does it take to suppress feelings of boredom?"). Scores on the RSME are based on the mean of all seven scales and range from 0 to 150.

Emotion Regulation Task

The emotion regulation task (based on Ochsner et al., 2002 and adopted from Modinos, Ormel, & Aleman, 2010[a]) was programmed in Eprime version 1.2, running under windows XP on a HP Compaq Desktop PC (Intel Core 2 processor, 17 inch 60 Hz monitor). Stimuli were back-projected onto a screen located behind the scanner. Participants viewed the screen through an angled mirror attached to the head coil.

Because the task was performed on two occasions, two versions of the task were designed using two different stimulus sets. All other task parameters were equal in both versions. The order in which the participants performed the tasks was counterbalanced between individuals within each group. Each stimulus set contained 66 pictures from the International Affective Picture System (IAPS; Lang et al., 1997). Twenty-two neutral pictures (valence *M* = 5.10, *SD* = 0.29, arousal *M* = 3.26, *SD* = 0.53 for both sets) and 44 negative pictures (valence *M* = 2.54, *SD* = 0.49, arousal *M* = 5.83, *SD* = 0.66 for both sets) were selected, based on normative scores (Lang, Bradley, & Cuthbert, 2008). Negative pictures depicted complex scenes of burn victims, funerals, and interpersonal violence.

The task used an event-related design with three main conditions: attend neutral (attentively viewing of a neutral picture), attend negative (attentively viewing of a negative picture), and reappraise (reinterpretation of a negative picture). All stimuli were presented centrally on a black background. Each trial of the task started with a picture for 2 s with the instruction VIEW in white letters underneath the picture. During this period, the participants were instructed to view the picture and to experience their emotional response to the picture. Next, the instruction VIEW was replaced by either ATTEND (for neutral or negative pictures) or REAPPRAISE (only for negative pictures) while the picture remained on the screen for a period of 4 s. In case of ATTEND, participants were instructed to continue to attend to the picture and experience any feelings arising naturally. In case of REAPPRAISE, participants were instructed to reappraise the content of the picture so that it no longer evoked a negative response. Then, an empty black screen replaced the picture for 2 s, during which the participants were instructed to keep on attending to or regulating any lingering feelings. The following 3 s, a four-point rating scale was presented and the participants rated their current strength of negative affect by pressing one of four buttons (1 = weak to 4 = strong). The trials ended with the word RELAX for 4 s. Trials were separated by an inter-trial interval (black screen) of 500 ms duration. A total of 66 trials of 15 s were presented with 22 trials for each condition. Clusters of 6-9 trials were interleaved with eight 20 s fixation blocks (i.e., a white fixation cross presented centrally on a black background). The order of trial conditions (attend

neutral, attend negative, reappraise) was randomized and the negative pictures were randomly divided over both negative conditions (attend negative and reappraise). The task was presented in two runs of approximately 10 minutes each.

fMRI Data Acquisition

Imaging data were collected on a GE Signa HDxt 3.0-Tesla MRI-scanner with an 8-channel head coil (General Electric, Milwaukee, Wisconsin) at the VU University Medical Center. Functional images were acquired with 40 slices in ascending order using a T2*-weighted echo planar imaging (EPI) sequence (repetition time (TR) = 2100 ms, echo time (TE) = 30 ms, flip angle (FA) = 80°, field of view (FOV) = 22.4 x 22.4 cm, voxel size = 3.5 x 3.5 x 3 mm). A total of 584 volumes were obtained in two runs of 292 volumes. A T1-weigthed anatomical scan with 172 slices was acquired for co-registration and normalization (TR = 8.2 ms, TE = 3.2 ms, FA = 12°, FOV = 25.6 x 25.6 cm, voxel size = 1 x 1 x 1 mm).

Questionnaire and Behavioral Data Analysis

Statistical analysis of the questionnaires and behavioral data was carried out with PASW Statistics 18.0 (Chicago: SPSS Inc., IL). Independent *t*-tests were carried out to compare the groups on descriptive variables, questionnaire scores and neuropsychological tests. The CES-D and PPVT showed skewed distributions, which violated the assumption of normality. Therefore, we applied non-parametric Mann-Whitney *U* tests to compare the groups on these variables. Fatigue ratings measured with the RSME were analyzed in a mixed repeated-measures ANOVA with *time of assessment* (T0, T1, T2) and *manipulation* (fatigue, control) as within-subjects factors, and *group* (fatigue, control) as between-subjects factor.

Reported strength of negative affect was compared in a condition (attend neutral, attend negative, reappraise) x manipulation (fatigue, control) x group (fatigue, control) mixed repeated-measures ANOVA.

fMRI Data Analysis

Imaging data were analyzed with Statistical Parametric Mapping (SPM8; www.fil.ion.ucl.ac.uk/spm). Preprocessing comprised reorienting, followed by realignment and unwarping, slice time correction, co-registration, normalization into MNI standard space, reslicing to voxels of 3 x 3 x 3 mm, and spatial smoothing with an 8 mm full width at half maximum (FWHM) isotropic Gaussian kernel.

At first level, we modeled fixation, attend neutral, attend negative and reappraise trials. A boxcar function was convolved with the hemodynamic response function for the 4 s period during which participants attended to or reappraised the picture. A high-pass filter of 128 s cutoff was applied to remove low frequency noise. In case of motion artifacts (i.e., > 0.5 mm movement between scans), additional regressors were computed and added to the model as regressors of no interest. For each participant in each session, first level contrasts were computed to assess the BOLD response to negative pictures (attend negative > attend neutral) and reappraisal (reappraise > attend negative). The following analyses were performed for each of the two conditions.

The first level contrasts were entered into second level mixed ANOVAs with group (2 levels) as independent factor and manipulation (2 levels) as dependent factor. For the main effect of condition, significance level was thresholded at $p < .05$ following a Family Wise Error rate (FWE) correction for multiple comparisons. For group and manipulation differences, the FWE correction was too conservative and we therefore used a Monte Carlo simulation of brain volume (Slotnick et al., 2003) to obtain appropriately corrected results. Assuming an individual voxel type I error of $p < .005$, a cluster extent of 30 contiguous resampled voxels (3 x 3 x 3 mm) was required to correct for multiple comparison at $p < .05$.

Habitual reappraisal. To evaluate effects of fatigue in the context of spontaneous emotion regulation, we additionally investigated associations between habitual reappraisal and the BOLD response to viewing negative pictures. The individual scores on

the ERQ reappraisal subscale were added to the model as covariate of interest, which we allowed to interact with group. Associations between ERQ reappraisal scores and the BOLD response to viewing negative stimuli were investigated using Pearson's product-moment correlations.

RESULTS

Sample Characteristics

Sample characteristics are summarized in Table 1. The fatigue group scored significantly higher on all subscales of the CIS and reported higher levels of depression ($p < .001$). Moreover, the control group made significantly more habitual use of reappraisal strategies compared to the fatigue group ($p = .002$). Both groups scored equally on non-verbal and verbal intelligence and all other neuropsychological tests (the corrected alpha for 15 tests with a mean correlation coefficient of $\rho = .12$ corresponded to .0047214; http://www.quantitativeskills.com/sisa/calculations/bonfer.htm).

Table 1

Sample Characteristics

		Fatigue group	Control group	*p*
N		26	26	
Age		21.8 (2.3)	21.6 (1.9)	.686
CIS score	total	85.1 (15.5)	39.1 (9.7)	<.001
	fatigue severity	38.4 (7.2)	16.6 (5.2)	<.001
	concentration	20.2 (6.5)	10.1 (3.9)	<.001
	motivation	12.8 (4.1)	6.3 (1.7)	<.001
	physical activity	13.8 (4.4)	6.2 (3.6)	<.001
CES-D		14.0 (7.5) [a]	4.2 (3.5)	<.001
ERQ reappraisal		4.1 (0.8)	4.8 (0.8)	.002
ERQ suppression		3.0 (1.2)	2.5 (1.1)	.128
Hb (nmol/l)		8.4 (1.0) [b]	8.5 (0.7) [a]	.699
RAVEN		23.3 (3.1)	22.9 (3.8)	.633
PPVT		108.9 (7.4)	109.8 (6.8)	.898
LDST		45.4 (4.4)	46.5 (4.9)	.497
CST		6.4 (4.8) [a]	4.1 (3.5)	.057
STROOP interference		29.1 (9.2) [a]	22.7 (7.6)	.010

Note. [a] Data of 1 person is missing (*N*=25); [b] Data of 2 persons is missing (*N*=24).

Manipulation Check

The effect of the manipulation on fatigue ratings is illustrated in Figure 1. A significant interaction between time of assessment and manipulation ($F(2,100)=21.34$, $p < .001$) indicated that fatigue ratings increased more in the fatigue session compared to the control session. Post hoc t-tests confirmed that fatigue ratings differed significantly between sessions at T1 (i.e., higher fatigue ratings after the fatigue manipulation compared to the control manipulation; $t(51) = 6.67$, $p < .001$, $r = .34$) and not at T0 and T2 ($t < 0.95$, $p > .346$, $r < .14$). Other significant effects showed that overall, fatigue increased from T0 to T2 ($F(2,100) = 82.54$, $p < .001$), fatigue ratings were higher in the fatigue session compared to the control session ($F(1,50) = 14.49$, $p < .001$), and the fatigue group had higher subjective fatigue compared to the control group ($F(1,50) = 10.88$, $p = .002$). A significant interaction between group and time of assessment ($F(2,100) = 6.61$, $p = .002$)

indicated that across both sessions, the fatigue group showed a steeper increase in fatigue ratings from T0 to T2 compared to the control group. No other significant effects were observed ($F < 0.94$, $p > .396$).

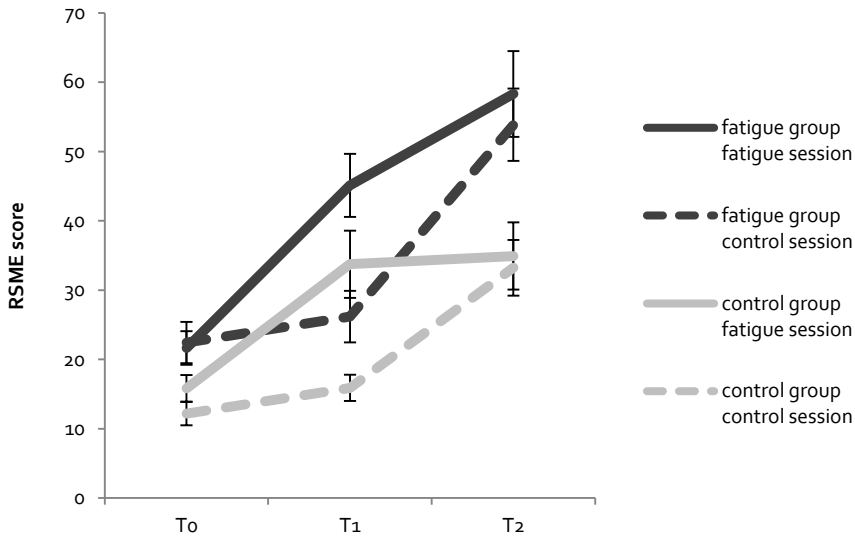


Figure 1. Fatigue scores measured by the Rating Scale of Mental Effort (RSME) at baseline (T0), after the manipulation (T1) and after scanning (T2). Dark gray represents the fatigue group and light gray represents the control group with solid lines for the fatigue session and dashed lines for the control session. Error bars show standard error of the mean (SEM). The scores differed significantly between sessions only at T1.

Behavioral Results

Mean strength of negative affect ratings are summarized in Table 2. The mixed repeated measures ANOVA showed that regardless of manipulation or group, the negative emotion experience differed between all conditions ($F(2,100) = 313.85$; $p < .001$; $r = .87$). Negative affect increased after attend negative compared to attend neutral trials (mean difference = 1.55, SE = 0.07; $p < .001$), which declined after reappraisal trials (mean difference between reappraise and attend negative = 0.62, SE = 0.06; $p < .001$). These results confirmed that negative pictures evoked negative affect and that reinterpretation of negative pictures successfully reduced the experience of negative emotion. There were

no effects of manipulation or of group on negative affect ratings ($F < 1.77$; $p > .190$; $r < .19$ for all other comparisons).

Table 2

Reported Strength of Negative Affect: M(SD)

	Fatigue session		Control session	
	Fatigue group	Control group	Fatigue group	Control group
Attend neutral	1.12 (0.3)	1.20 (0.4)	1.17 (0.2)	1.01 (0.3)
Attend negative	2.69 (0.5)	2.70 (0.6)	2.74 (0.6)	2.55 (0.8)
Reappraise	2.13 (0.5)	2.08 (0.5)	2.10 (0.5)	1.88 (0.7)

Note. Reported strength of negative affect increased for negative pictures relative to neutral pictures, and decreased again after reappraisal. There were no effects of fatigue group or session.

fMRI Results

Negative versus neutral pictures. The attend negative minus attend neutral contrast revealed activations representing processing of negative emotion. The results across groups and sessions are summarized in Table 3. Large activated clusters covered bilateral insula, thalamus, temporal lobe, prefrontal cortex (PFC) and parietal areas, with the most prominent peak activations in bilateral fusiform gyrus, anterior cingulate cortex (ACC), bilateral dorsolateral PFC (DLPFC), and inferior parietal lobule (IPL).

Effects of fatigue group. Across sessions, the contrast *fatigue group > control group* revealed a cluster that covered the right amygdala (Figure 2A; see Table 4 for peak activations of this cluster). The fatigue group showed stronger amygdala activation compared to the control group in response to negative pictures (Figure 2B). The reversed contrast (*control group > fatigue group*) revealed no significantly activated clusters.

In a post hoc analysis, we investigated whether the group difference in amygdala response was accompanied by differences in functional associations with other brain areas. To this end, we extracted the mean parameter estimates of the activated cluster and replicated the mixed ANOVA with these values added as covariate of interest. We

observed two PFC clusters showing a group x covariate interaction, but this effect appeared to be driven by outliers (non-parametrical tests, i.e., Spearman's rho, showed no correlations).

To evaluate whether the difference in amygdala response was due to increased amygdala response to neutral stimuli (which is sometimes found in control groups: Hall et al., 2008), we compared the groups on the Attend neutral > Fixation contrast. Analyses at whole brain level as well as ROI analyses with the right amygdala cluster as ROI, revealed no differences between the groups (ROI analysis: $t(50) = 1.07$, $p = .290$). Increased amygdala response in fatigued students was thus attributable to negative pictures and not to changes in the processing of neutral pictures.

Table 3

Brain regions that are activated when viewing negative pictures (attend negative > attend neutral)

Peak activation area	BA	x	y	z	*z*	k
Fusiform Gyrus	19	48	-73	-8	> 8	1324
Culmen		45	-52	-23	> 8	
Middle Occipital Gyrus	18	33	-88	-5	> 8	
Inferior Occipital Gyrus	19	-48	-76	-2	> 8	1057
Fusiform Gyrus	37	-42	-49	-20	> 8	
Cerebellum (Uvula)		-12	-73	-26	5.86	
Cingulate Gyrus	32	-6	26	37	> 8	2453
Middle Frontal Gyrus	9	45	23	25	> 8	
Middle Frontal Gyrus	9	51	14	34	> 8	
Inferior Parietal Lobule	40	-42	-34	43	7.74	611
Inferior Parietal Lobule	40	-36	-52	52	7.73	
Precuneus	7	0	-61	34	7.22	464
Posterior Cingulate	23	-3	-49	25	7.11	
Precuneus	7	3	-58	43	6.89	
Superior Parietal Lobule	7	39	-52	52	7.2	432
Inferior Parietal Lobule	40	48	-31	40	5.73	
Thalamus		-18	-34	1	7.19	1280
Caudate (body)		12	8	10	7.01	
Thalamus		9	-7	7	6.86	
Precentral Gyrus	6	-54	8	34	6.62	588
Precentral Gyrus	9	-48	26	31	6.58	
Claustrum		-30	20	1	6.51	
Precentral Gyrus	6	-27	-4	52	6.06	111
Middle Frontal Gyrus	6	-36	5	58	5.57	
Middle Frontal Gyrus	10	-30	53	7	5.49	38

Note. Activations are thresholded at $p < .05$, FWE corrected. Coordinates are in MNI space. BA = Brodmann Area.

Table 4

Effects of fatigue on the processing of negative pictures (1st level contrast: attend negative > attend neutral)

Peak activation area	BA	x	y	z	*z*	k
Group main effect: fatigue > control						
Parahippocampal Gyrus	28	24	-16	-17	3.14	53
Substania Nigra		15	-16	-17	3.14	
Manipulation main effect: control > fatigue						
Middle Occipital Gyrus	18	-21	-85	-14	3.60	209
Inferior Occipital Gyrus	18	-36	-85	-11	3.33	
Inferior Occipital Gyrus	18	-33	-85	10	3.28	
Superior Occipital Gyrus	19	33	-76	22	3.10	37
Precuneus	7	27	-70	28	2.85	
Middle Temporal Gyrus	39	42	-76	13	2.84	
Group x manipulation interaction						
Globus Pallidus		21	-7	1	3.38	54
Thalamus (Pulvinar)		15	-25	4	2.74	
Cerebellum (Declive)		15	-70	-26	2.98	36
Cerebellum (Declive)		3	-67	-23	2.95	

Note. Activations are thresholded at $p < .005$, corrected for magnitude of k = 30, and inclusively masked by the main effect of condition ($p < .05$, uncorrected). Coordinates are in MNI space. BA = Brodmann Area.

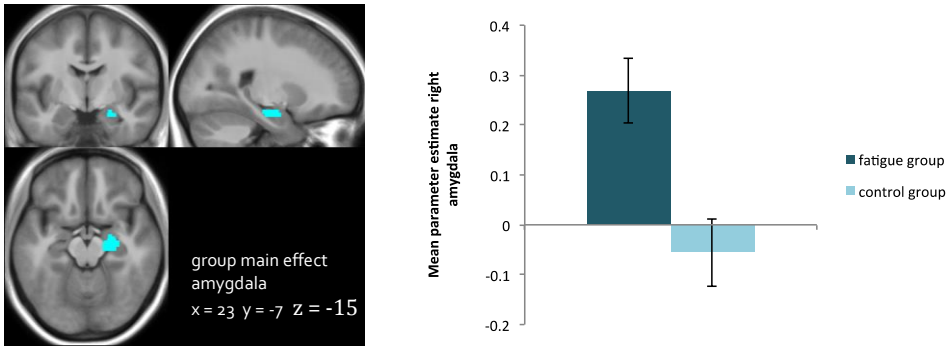


Figure 2. Main effect of group (fatigue group > control group) in response to attend negative > attend neutral pictures overlayed on a mean anatomical image of all participants. A cluster including the right amygdala was activated more by the fatigue group across both sessions (left). The graph illustrates the mean parameter estimates within the activated amygdala cluster with error bars showing SEM (right).

Effects of fatigue manipulation. The contrast *control session* > *fatigue session* revealed two large clusters that covered part of the left and right extrastriate visual cortex, which in the right hemisphere extended into the middle temporal gyrus and precuneus (Table 4). No activated clusters were observed for the opposite contrast.

Group and manipulation interactions. Significant group x manipulation interactions were observed in two large clusters (Table 4), covering the globus pallidus, thalamus and areas in the cerebellum. The control group showed decreased activation following the fatigue manipulation, whereas the fatigue group showed increased activation compared to the control manipulation in these areas. This was confirmed by post hoc t-tests showing significantly decreased activation in the control group ($t < -2.45$, $p_{corrected} < .05$ for both comparisons) and a trend for an increase in these areas in the fatigue group ($t > 1.79$, $p_{corrected} < .080$ for all comparisons).

Habitual reappraisal. Areas that showed group interactions in the correlation between ERQ reappraisal and BOLD response are presented in Table 5. The control group showed positive correlations in areas that are typically associated with reappraisal (i.e., dorsomedial PFC [DMPFC], right orbitofrontal cortex [OFC], and bilateral DLPFC; Ochsner and Gross, 2005; Pearson's r across all four regions = .70, $p < .001$), and a negative correlation in the left amygdala ($r = -.51$, $p < .01$). The fatigue group showed opposite effects; this group was characterized by a trend for negative correlations in cognitive reappraisal areas ($r = -.43$, $p = .030$, not significant after a Bonferroni corrected p-value of .0134717, adjusted for a mean r of .05; http://www.quantitativeskills.com/sisa/calculations/bonfer.htm) and a positive correlation with left amygdala ($r = .63$, $p < .001$). The differential correlations are illustrated in Figure 3.

In post hoc analyses, we investigated between-subjects functional connectivity by examining correlations between mean BOLD response in the left amygdala cluster and the BOLD response in the four (spontaneous) reappraisal clusters. In the control group, amygdala activation correlated negatively with bilateral DLPFC (BA9), which was

significant in the left DLPFC (r = -.62, p = .001) and a trend in the right DLPFC (r = -.39, p = .048; the Bonferroni corrected p-value for 8 tests adjusted for a mean correlation coefficient of r = .34 corresponded to 0.0126745). No correlations were observed in the fatigue group or in other PFC areas in both groups (r > -.20; p > .334 in the control group and r > -.23; p > .263 in the fatigue group).

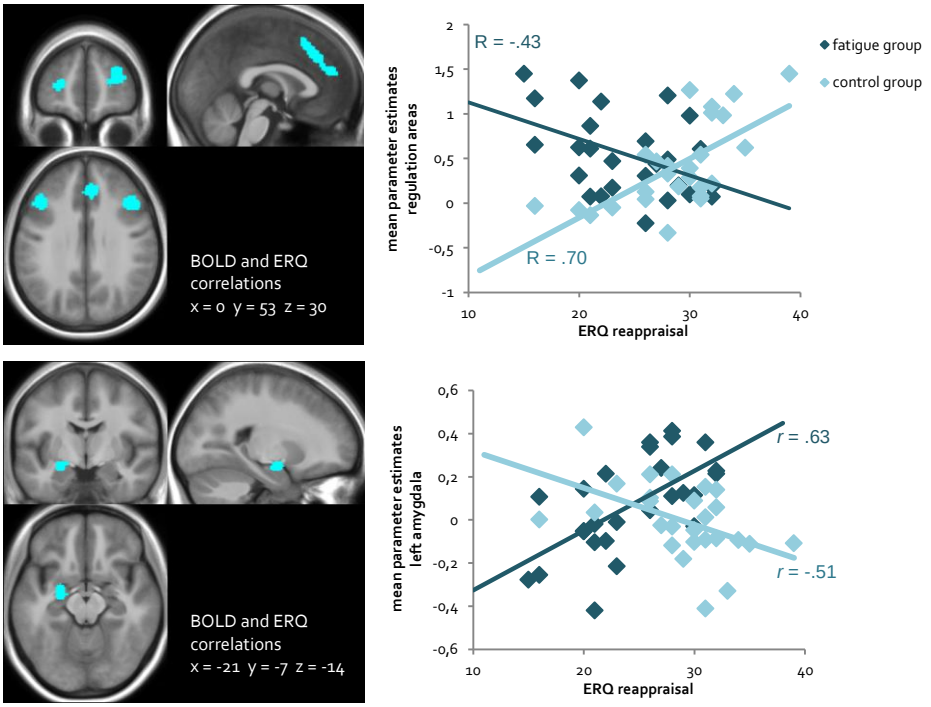


Figure 3. Correlations between habitual reappraisal (i.e., ERQ reappraisal scores) and BOLD response to viewing of negative pictures (i.e., attend negative > attend neutral). Images on the left side represent areas in which the correlation interacted with group overlaid on the mean anatomical image of all participants. Coordinates are in MNI space, BOLD = blood oxygenation-level dependent. Scatterplots on the right side illustrate the correlations (including Pearson's r) between the mean parameter estimates of the areas presented on the left side and ERQ reappraisal scores. Dark turquoise represents the fatigue group and light turquoise represents the control group.

Table 5

Brain regions showing group interactions in the associations between self-reported reappraisal use and BOLD response in the attend negative > attend neutral contrast

Peak activation area	BA	x	y	z	*z*	k
Attend negative > Attend neutral						
Cingulate Gyrus	32	9	23	40	4.32	388
Superior Frontal Gyrus	6	18	17	61	3.94	
Medial Frontal Gyrus	32	-9	17	46	3.72	
Inferior Frontal Gyrus	47	36	29	-8	3.65	60
Inferior Frontal Gyrus	13	42	23	4	3.23	
Inferior Frontal Gyrus	13	39	29	13	2.88	
Middle Frontal Gyrus	9	-39	26	25	3.59	66
Middle Frontal Gyrus	9	-48	26	31	3.46	
Precentral Gyrus	9	45	23	34	3.37	99
Amygdala		-21	-7	-14	3.53	38

Note. Activations of condition main effects are thresholded at $p < .05$, FWE corrected. Manipulation effects are thresholded at $p < .005$, corrected for magnitude of k = 30, and inclusively masked by the main effect of condition ($p < .05$, uncorrected). Coordinates are in MNI space. BA = Brodmann Area.

Reappraisal of negative pictures. The reappraise minus attend negative contrast revealed activations in temporal lobe, parietal and PFC areas. Activated clusters covered areas that are consistent with the reappraisal literature (Ochsner and Gross, 2005) such as dorsolateral and dorsomedial PFC (DLPFC, DMPFC), anterior cingulate cortex (ACC) and bilateral orbitofrontal cortex (OFC). Peak activations are presented in Table 6.

Effects of fatigue group / manipulation. No main effects of fatigue group or interactions involving fatigue group were found. A cluster in the right DLPFC showed increased activation after the fatigue manipulation compared to the control manipulation (Table 6). We found no areas showing the opposite effect.

Table 6

Brain regions that are activated during reappraisal of negative pictures (reappraise > attend negative)

Peak activation area	BA	x	y	z	*z*	k
Condition main effect (all groups and sessions)						
Middle Temporal Gyrus	39	-51	-61	28	> 8	547
Inferior Parietal Lobule	39	-48	-64	43	> 8	
Superior Frontal Gyrus	6	-12	17	61	> 8	2923
Inferior Frontal Gyrus	47	-39	20	-11	> 8	
Middle Temporal Gyrus		-54	-37	-5	> 8	
Superior Temporal Gyrus	39	60	-58	31	> 8	396
Supramarginal Gyrus	40	63	-52	22	> 8	
Middle Temporal Gyrus	21	54	-31	-5	> 8	191
Middle Temporal Gyrus	21	48	5	-32	7.08	460
Inferior Frontal Gyrus	47	45	23	-11	6.97	
Inferior Frontal Gyrus	9	57	20	16	6.91	
Middle Frontal Gyrus	6	42	17	46	6.22	118
Cingulate Gyrus	24	0	-19	37	4.84	37
Manipulation main effect: fatigue > control						
Middle Frontal Gyrus	9	48	32	28	3.08	32
Middle Frontal Gyrus	46	51	35	19	3.06	
Middle Frontal Gyrus	9	48	20	31	2.69	

Note. Activations of condition main effects are thresholded at $p < .05$, FWE corrected. Manipulation effects are thresholded at $p < .005$, corrected for magnitude of k = 30, and inclusively masked by the main effect of condition ($p < .05$, uncorrected). Coordinates are in MNI space. BA = Brodmann Area.

DISCUSSION

The goal of the present fMRI study was to investigate the relation between fatigue in medical students and changes in the processing and regulation of negative emotion. Our main hypothesis referred to enhanced emotion reactivity in fatigued students due to reduced cognitive control. To this end, we compared chronically fatigued medical students to their non-fatigued peers after a fatigue-inducing manipulation and a control manipulation. While we observed no effects of fatigue at the behavioral level, we found prominent group differences in neural activation that were in favor of our hypothesis. As

opposed to their non-fatigued peers, fatigued students showed enhanced right amygdala response to negative stimuli, reversed correlations between habitual reappraisal and neural activation of the left amygdala as well as reappraisal areas, and no direct functional connection between left amygdala and DLPFC when viewing negative stimuli. During instructed reappraisal, no group differences were observed.

Across group and session, we found a significant increase in reported negative affect after attentively viewing negative compared to neutral pictures. This decreased again after reappraisal of negative pictures. These findings indicate that negative pictures induced the experience of negative emotion and that participants in both groups were successful at reducing negative affect by cognitive reappraisal. The fMRI data showed that attentively viewing negative pictures increased activation of a wide range of brain areas that also covered bilateral amygdala and insula. These are areas that are generally associated with emotion recognition and the production of affective state and behavior (Phillips, Drevets, Rauch, & Lane, 2003). Viewing negative pictures additionally increased activation of regulation areas, such as dorsal ACC and bilateral DLPFC (Ochsner & Gross, 2005; Phillips et al., 2008; Mary L Phillips et al., 2003). It is possible that the negative stimuli induced a certain degree of automatic regulation, which has been demonstrated in other studies (Drabant et al., 2009; Rive et al., 2013). Reappraisal of negative pictures was associated with increased activation of a fronto-parietal network that predominantly included prefrontal areas consistent with prior emotion regulation findings, such as DLPFC, ACC and OFC (Goldin et al., 2008; Modinos et al., 2010[a], 2010[b]; Ochsner et al., 2002).

The main group difference found in the present study involved activation of the amygdala in response to negative pictures. Specifically, chronically fatigued students activated the right amygdala more than non-fatigued students on attend negative compared to attend neutral trials. Consistent with the commonly reported increase in irritability and enhanced negative mood by fatigued individuals (Dahl, 1999; Leavitt & DeLuca, 2010; Oginska & Pokorski, 2006; Owens, 2001; Ream & Richardson, 1996), this

finding suggests that fatigued students are more emotionally reactive when it comes to negative stimuli. In parallel, the fatigued students reported significantly higher levels of depression. It is conceivable that enhanced processing of negative emotion underlies the increase in depressed mood. Furthermore, stronger amygdala responsiveness to negative affective stimuli is also found in depression (e.g., Sheline et al., 2001; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007), anxiety (Beesdo et al., 2009; Phan, Wager, Taylor, & Liberzon, 2002) and sleep deprivation (Yoo, Gujar, Hu, Jolesz, & Walker, 2007). Collectively, these studies associated enhanced amygdala reactivity with reduced regulation by PFC control regions.

Individuals commonly engage in spontaneous emotion regulation (Gross & John, 2003) and the extent to which spontaneously regulation occurs can depend on habitual use of reappraisal strategies in everyday life (Drabant et al., 2009). Therefore, we additionally evaluated group differences in associations between daily use of reappraisal strategies and brain activation during exposure to negative stimuli. First, it should be noted that the fatigued students scored significantly lower on habitual reappraisal as measured with the ERQ. Thus, based on their own reports, they made less frequent use of reappraisal strategies in their everyday lives. As for correlations with habitual reappraisal and neural response to negative pictures, we observed group interactions in DMPFC, right OFC, bilateral DLPFC, and left amygdala. Consistent with Drabant et al., (2009), in the group of non-fatigued students, habitual reappraisal was positively correlated with PFC areas that are typically associated with reappraisal (Ochsner & Gross, 2005, 2008; Urry et al., 2006), and negatively with the left amygdala. This aligns well with earlier indications for automatic regulation (Abler, Erk, Herwig, & Walter, 2007; Erk, Abler, & Walter, 2006) and suggests that non-fatigued students with higher habitual reappraisal engage more in spontaneous emotion regulation. In addition, the non-fatigued students showed a negative correlation between DLPFC and the left amygdala, which emphasizes the notion of automatic regulation of amygdala by the PFC in this group. The group of fatigued students, however, showed atypical correlations in these areas. Namely, fatigued students who reported more habitual use of reappraisal showed stronger left

amygdala responsiveness and weaker PFC activation. A plausible explanation for this atypical effect is that fatigued students who are more responsive to negative stimuli would be more urged to regulate their emotions on a daily basis. Still, this does not explain the trend for a negative relation between habitual reappraisal and PFC activation. It implies that fatigued students who make more habitual use of reappraisal are less likely to engage these regulation areas. Yet, we observed no functional connectivity in this group between PFC and left amygdala, suggesting that engagement of these PFC regions did not account for effective automatic regulation in chronically fatigued students.

Our findings showed different effects between right and left amygdala. Although there is no general consensus on whether and to what extent the right and left amygdala are functionally distinctive, there are some indications for functional differentiation. First of all, activation of the amygdala is most often left lateralized, particularly for stimuli of negative valence (Baas, Aleman, & Kahn, 2004). Furthermore, prominent functional differences may pertain to the speed and extent of emotion processing. For instance, Gläscher & Adolphs (2003) proposed that the processing in the right amygdala is fast, automatic, and global (see also Zald, Lee, Fluegel, & Pardo, 1998), whereas the left amygdala is activated later and involved in more detailed and sustained emotional processing (Baas et al., 2004). This could explain why in the current study the left and not the right amygdala was the subject of (in this case: spontaneous) regulation. Stated differently, the present results are aligned with this model of amygdala lateralization by showing that PFC regulation involved the left amygdala, whereas emotion reactivity unrelated to PFC regulation involved the right amygdala. Moreover, regulation of the left amygdala is in line with prior studies that showed left lateralized effects of instructed (Modinos et al., 2010[a], 2010[b]) as well as spontaneous regulation (Drabant et al., 2009).

Effects of the fatigue manipulation were observed both during attentively viewing and during reappraisal of negative pictures. When viewing negative pictures, activation of regions implicated in visual-spatial processing (extrastriate visual areas and right

precuneus) decreased after the fatigue manipulation compared to the control manipulation. Accordingly, the cognitive fatigue induction might have affected visual attention, which corresponds to prior studies showing that induced fatigue can reduce attentional processes (Boksem et al., 2005; van der Linden & Eling, 2006). During instructed cognitive reappraisal, the fatigue manipulation resulted in enhanced activation of the right DLPFC. Increases in PFC areas can be an indication of increased neural effort (Park & Reuter-Lorenz, 2009) in response to any kind of cognitive challenge. This result is in agreement with prior fatigue research that associated fatigue with increased neural activation (Chaudhuri & Behan, 2000; Chaudhuri & Behan, 2004; Cook et al., 2007).

A final observation comprised interactions between fatigue group and fatigue manipulation when viewing negative pictures. Fatigued and non-fatigued students responded differently to the fatigue manipulation in terms of activation in the thalamus and globus pallidus, and the cerebellum. In these areas, non-fatigued students showed significantly decreased activation following the fatigue manipulation, whereas fatigued students showed a slight increase in activation. These areas have all previously been associated with emotion processing (Anand, Malhotra, Singh, & Dua, 1959; Colibazzi et al., 2010; Goldin et al., 2008; Habel, Klein, Kellermann, Shah, & Schneider, 2005; LaBar & Cabeza, 2006; Schmahmann, 2000; Schutter & van Honk, 2005, 2009). Activation of the thalamus and globus pallidus together has been directly related to emotional arousal (Colibazzi et al., 2010). Moreover, both the cerebellum and the thalamus have regulatory properties, as the cerebellum is involved in emotion regulation (Schutter & van Honk, 2005, 2009) and the thalamus can modulate cortical activation by controlling the transmission of (sensory) information (Heilman, 2002). The present results thus suggest a differential effect of the cognitively demanding fatigue manipulation on emotional arousal or spontaneous modulatory processes in fatigued and non-fatigued students.

In contrast to our expectation, we observed no group differences in brain activation during instructed emotion regulation. Based on clinical studies (Deluca, Genova, Hillary, & Wylie, 2008; Kohl, Wylie, Genova, Hillary, & Deluca, 2009; McAllister et al., 2001), we

expected that fatigued individuals would require increased mental effort during explicit reappraisal. The current findings indicate that fatigued and non-fatigued students are equally able to employ cognitive reappraisal strategies when they are instructed to do so. As negative affect ratings decreased after instructed reappraisal in both groups, these strategies appeared to be effective.

To maximize the emotional response and to minimize confounding factors, the present study included only females from a population that is relatively homogeneous in terms of motivation, intelligence and educational background (i.e., medical students). The downside of this approach is that the results cannot easily be generalized across males or individuals with other educational backgrounds.

To conclude, the present study reports that female medical students with prolonged fatigue are characterized by stronger emotional reactivity to negative stimuli. This may in part be based on differences in spontaneous and habitual cognitive reappraisal. In other words, enhanced reactivity to negative stimuli and atypical automatic regulation may be at the core of fatigue vulnerability. Dysfunctional emotion regulation is thus a promising target for future intervention research. Moreover, the current result raises the question whether fatigued individuals are also more reactive to positive emotional stimuli or to stimulation in general. This is of particular relevance for modern society in which individuals are constantly connected to others and continuously encountered with emotive stimulation, such as text messages, news reports and advertisements.

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