Cognition and the Middle-Aged Brain

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Chapter 5

Cortisol and induced cognitive fatigue: effects on memory activation in healthy males

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ABSTRACT

We investigated the relationship between individual differences in acute fatigue and endogenous cortisol changes elicited by the sustained performance of cognitively demanding tasks (fatigue condition). Healthy males provided salivary cortisol measurements and subjective fatigue ratings, and were scanned (functional magnetic resonance imaging) during memory encoding and recognition tasks in fatigue and control conditions. A group of 15 'responders' (showing a significant cortisol increase) were compared to 12 'non-responders'. Responders showed higher subjective fatigue and reduced encoding and recognition activation than non-responders in the fatigue condition. An interaction in the right hippocampus during encoding reflected decreased activation in responders, but somewhat increased activation in non-responders in the fatigue compared to control condition. Moreover, decreased hippocampal activation in responders was associated with increased subjective fatigue. Findings are consistent with a central role for the hippocampus in differences between responders and non-responders and also implicate the right hippocampus in individual differences in induced cognitive fatigue effects.

* This chapter is based on data from fMRI study 1.

INTRODUCTION

Fatiguing situations are commonly encountered in daily life and are associated with negative consequences, such as an increased risk of accidents. Cognitive fatigue can be defined as arising from the 'prolonged performance of cognitively demanding tasks requiring sustained mental efficiency' (Lorist, 2008). This acute state of induced cognitive fatigue is associated with increased subjective fatigue, behavioural performance decrements and decreased amplitude of event-related potential components (Boksem et al., 2006; Kato et al., 2009; Lorist, 2008; Lorist et al., 2005; van der Linden et al., 2003). Cognitively demanding tasks have also been shown to elicit a cortisol stress response in some individuals (Bohnen et al., 1990; Kukolja et al., 2008). Yet, little is known about the differences between individuals who show a significant cortisol response (called responders) and individuals who do not (non-responders) in relation to induced cognitive fatigue. Relatively more is known about differences between responders and non-responders in the context of psychological stress paradigms and the effect of cortisol on memory performance, although insight into the neural correlates of memory effects is limited (van Stegeren, 2009). Therefore, in the present study, we aimed to determine differences between responders and non-responders in an induced fatigue condition (compared to a control condition) in terms of subjective fatigue, memory performance and memory-related brain activation (using functional magnetic resonance imaging; fMRI).

Cortisol, an adrenal corticosteroid, is released as a result of activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol secretion shows a strong circadian rhythm (levels typically increase in the hour following awakening and then decline over the rest of the day) as well as short-term increases in response to acute stress. Cortisol has been linked to fatigue in studies showing reduced cortisol levels in chronic fatigue syndrome (CFS) (Jerjes et al., 2005; Nater et al., 2008; Silverman et al., 2010) and vital exhaustion (Nicolson and van Diest, 2000); a blunted cortisol response to wakening in relation to fatigue, burnout and exhaustion in populations both with and without CFS (Chida and Steptoe, 2009); low waking cortisol levels and a flatter diurnal slope of cortisol secretion in relation to current and future fatigue in a sample of healthy adults (Kumari et al., 2009); and CFS symptom alleviation with cortisol administration (Rimes and Chalder, 2005). These studies point to a relationship between chronic fatigue complaints and HPA axis dysregulation. However, there is also some evidence linking acute task-induced fatigue and cortisol levels in healthy adults, with reduced task-induced subjective fatigue found following exogenous cortisol administration (Reuter, 2002; Tops et al., 2006). Hence, in the short-term (minutes to hours), cortisol is thought to elicit energy mobilisation and an associated decrease in perceived fatigue as part of a generally adaptive response called allostasis (McEwen, 2007). However, in the long-term (weeks to months), frequent high cortisol levels in association with chronic stress or 'allostatic load' (McEwen, 2007) may lead to downregulation of the HPA axis, reflected in decreased cortisol levels and reactivity, and ensuing chronic fatigue (Boksem and Tops, 2008).

In the current study, we focus on short-term effects in relation to the acute cortisol response to stress. However, the induced fatigue paradigm used in the present study differs somewhat from psychological stress paradigms commonly used to elicit an acute cortisol

response. Stress paradigms generally require demanding tasks to be performed under conditions of uncontrollability or social evaluative threat and are commonly completed in less than 30 min (Dickerson and Kemeny, 2004b). Studies using psychological stress paradigms have primarily focused on the relationship between the cortisol response and memory performance, consistently demonstrating enhanced memory as a result of an acute increase in cortisol during encoding, but memory impairment with increased cortisol during recognition (LaBar and Cabeza, 2006, Wolf, 2009, Roozendaal, 2002). To our knowledge, our study is the first to examine the effect of acute changes in endogenous cortisol levels elicited by the sustained performance of cognitively demanding tasks over a 3 h period in relation to both memory (performance and brain activation) and subjective fatigue. In the induced fatigue paradigm, fatigue is thought to arise when resources used for sustained cognitive effort are temporarily depleted (DeLuca, 2005). Therefore, we hypothesised that the initially positive, fatigue-alleviating effects of the cortisol response may become negative when prolonged task performance is required; the greater immediate mobilisation of resources in relation to the cortisol response may lead to greater subsequent resource depletion and, thus, greater induced fatigue effects during prolonged task performance. Indeed, a study examining the effect of prolonged task performance on subsequent cognition in relation to the elicited cortisol response, showed decreased attention in cortisol responders, although no effect was found on verbal memory performance (Bohnen et al., 1990). Brain activation changes in association with the cortisol response may be specifically expected in the hippocampus, amygdala and prefrontal cortex (PFC), areas thought to play a central role in the effects of cortisol on memory (Bangasser and Shors, 2010; de Quervain et al., 2000; Oei et al., 2007) and known to contain a high density of cortisol receptors (Gold et al., 2002).

We scanned male school teachers during encoding and recognition tasks in an induced fatigue condition and a control condition with the aim to determine differences between cortisol responders and non-responders. Responder and non-responder groups were necessarily created post-hoc based on cortisol response to the fatigue condition. We hypothesised that responders would show greater induced fatigue effects including increased subjective fatigue, impaired memory performance and an associated decrease in brain activation. We expected activation differences in relation to both study aims to be evident in the hippocampus, amygdala and PFC, in particular, and therefore used region of interest (ROI) analyses (in addition to whole brain analyses) to focus on these areas. Findings provide insight into the contribution of the cortisol response to individual differences in the effects of induced fatigue on subjective fatigue and memory.

METHOD

Participants

Participants were 27 healthy, right-handed, male school teachers aged 25 – 61 years. We restricted our sample population to males as gender differences in the effects of cortisol on both memory performance (Beck and Luine, 2010) and brain activation during stress tasks (Wang et al., 2005) have been found. Data from one participant were missing from the encoding task due to a scanner error. Volunteers who suffered significant past or present

physical or psychiatric illness, received medication (other than antihypertensives in two participants), or had MRI contraindications were excluded from participation. The study was approved by the local medical ethical committee at Maastricht University academic hospital. Participants gave informed consent prior to their (paid) voluntary participation.

Procedure

Participants completed a training session and two test sessions (control and fatigue conditions). The training session (administered in the week preceding the first test session) required participants to complete a neuropsychological test battery and practice the fMRI tasks in a dummy scanner to become familiarised with the scanning environment and minimise practice effects during the test sessions. Test sessions took place during two consecutive weekends at 0900, 1100 or 1300 h (time of day was kept constant within participants). Participants spent the first 1.5 h completing either the high cognitive demand fatigue manipulation or low demand control manipulation (administration order was randomised) outside the scanner. They were then scanned during the encoding and recognition tasks. Salivary cortisol levels were measured at four points, while subjective mood ratings were collected at three points throughout the sessions. The researcher operating the MRI scanner and instructing participants during scanning was blind to the manipulation the participants had just completed.

Neuropsychological testing

The visual verbal Word Learning Test (WLT) (Van der Elst et al., 2005) was administered as a measure of immediate and delayed memory recall and recognition, whereas the digit span (forward and backward) was administered as a test of short-term/working memory capacity (Lezak et al., 2004). General cognitive functions were tested using the letter digit substitution test (LDST) (van der Elst et al., 2006a) and the letter verbal fluency test (Van der Elst et al., 2006b). The Dutch version of the National Adult Reading test (Nelson, 1991) was administered as a measure of mental ability (intelligence) based on vocabulary.

Fatigue manipulation

During the fatigue condition, participants performed the following tasks: 2 and 3-N-back task (3 x 10 min), Stroop task with additional simultaneous auditory presentation of incongruent colour words (2 x 10 min), mental arithmetic (20 min), and brain teasers/puzzles (20 min). These tasks were selected for the high demands they place on a range of executive functions also subsequently involved in the scanning tasks. During the control condition, participants watched a documentary style DVD and/or read a magazine (e.g., the National Geographic) at their leisure.

fMRI encoding and recognition tasks

During the encoding (6 min) and recognition (16 min) tasks, 100 words were presented one- by-one on the screen. Words were divided equally into four semantic categories: food (F), animals (A), utensils/tools (U) and landscape features (L). During encoding, participants were instructed to indicate the category to which a word belonged by pressing the appropriate button, using the left- and right-hand middle and index fingers. The

categories were displayed at the bottom of the screen as each word was presented (as: F A U L). Participants were aware that they would subsequently be required to remember the encoding task words. During recognition, the same 100 'old' words were presented, plus an additional 100 'new' words. Participants were instructed to indicate with a button-press response whether they judged each word to be old or new, and how confident they were about this judgment. Response options therefore included: definitely old, probably old, probably new, and definitely new (displayed at the bottom of the screen as: Old 1 2 3 4 New). Encoding and recognition tasks were separated by a period of about 15 min, during which participants completed an unrelated task. In both tasks, words were presented in blocks of 8 stimuli followed by three null trials (consisting of a fixation point). Words were displayed in the center of the screen for 2500 ms, followed by a jittered inter-trial interval (500 - 1250 ms). In the training session, the tasks were practiced with 200 words from the categories sport, country, city and occupation.

Encoding task performance was examined in terms of overall accuracy and overall reaction time (RT). Recognition task performance was examined in terms of the number and corresponding RT of high and low confidence hits (correctly recognised old words) and correct rejections (correctly judged new words), and of misses (old words that were not recognised) and false alarms (new words judged to be old), regardless of confidence level. Additionally, recognition accuracy was examined using high confidence corrected recognition scores (hits minus false alarms). The corrected recognition score corrects for differences in the response criterion (participants who have a greater tendency to identify words as 'old' with high confidence during the recognition task will show more correctly judged 'old' words, but also more false alarms i.e. 'new' words incorrectly judged to be 'old').

Salivary cortisol

Saliva samples were collected upon arrival at the test session (time 0), between the fatigue manipulation and scanning session (time 1), approximately 40 min after entering the scanner (time 2) and at the end of the scan session (time 3). In relation to the fMRI tasks, samples were collected approximately 10 min prior to the encoding task (time 2 sample) and immediately following the recognition task (time 3 sample). An additional baseline sample was collected on a separate day (usually at the start of the training session) at the same time as the time 0 samples, to control for possible anticipation effects in relation to the test session. Saliva samples were collected using Salivettes (Sarstedt, the Netherlands) and stored at -20 ^{o}C until assayed. Salivary free cortisol levels were determined by a chemo-luminescence-assay (CLIA) with a high sensitivity of 0.16 ng/mL (IBL, Hamburg, Germany).

Area under the curve calculation: The change in salivary cortisol levels throughout the test sessions (in relation to baseline, calculated as the average of the time 0 sample and the additional training session baseline sample) was determined according to a formula for the calculation of the area under the curve with respect to increase (AUC_I) (Pruessner et al., 2003). The major advantages of using AUC_I values are that one score is calculated from the repeated measurements and the varying times between repeated measurements are taken into account.

Subjective ratings

The Dutch short visual analogue scale version of the Profile of Mood States (POMS) (Wald and Mellenbergh, 1990) was administered at three time points, coinciding with salivary cortisol measurement time 0, 1, and 3. The POMS fatigue (6 items: 0 = low fatigue, 100 = high fatigue) and vigour (5 items: 0 = low vigour, 100 = high vigour) subscales are recommended measures of the mood of energy and fatigue in investigations that are short in duration (e.g., a few hours, O'Connor, 2006) and the fatigue, vigour and tension (6 items: 0 = low tension, 100 = high tension) subscales have shown sensitivity to cortisol effects (e.g., Tops et al., 2006).

To assess longer-term feelings of mental fatigue, we administered the following four VAS questions during the training session: 'In the past week, to what degree has fatigue interfered with your ability to a) plan activities ahead of time, b) think clearly, c) think quickly, d) learn new things' (Dijkers and Bushnik, 2008).

MRI data acquisition

Scans were made in a 3 Tesla Philips whole body scanner (Philips Achieva, Philips Medical Systems, Best, the Netherlands). A body coil was used for RF transmission and an 8-element SENSE head coil (SENSE-factor 2) for signal detection. During encoding and recognition tasks, approximately 180 and 480 EPI scans were made, respectively (TR = 2.0 s, TE = 35 ms, number of slices = 32, image matrix = 64 x 64, voxel size = 4 x 4 x 3.5 mm). A T1-weighted anatomical scan was also acquired for anatomical reference and coregistration of the two test sessions (image matrix = 256 x 256, number of slices = 150, voxel size = 1 x 1 x 1 mm).

fMRI data analysis

SPM8 (Statistical Parametric Mapping: Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London) was used to preprocess the fMRI scans. Preprocessing steps included: slice time correction, realign and unwarp, coregistration (session 2 scans were coregistered to session 1 scans), spatial normalisation (MNI space, EPI template), and smoothing (FWHM 8 mm). Data were analysed in the context of the general linear model, using boxcar regressors convolved with the canonical hemodynamic response to model experimental trial blocks (consisting of 8 trials interspersed with three null trials) during encoding and recognition in each condition. In addition, motion parameters were included to correct for motion-related activation.

Encoding and recognition task activation contrasts were computed for each participant by contrasting activation during experimental blocks with null blocks (implicit baseline). Task-related activation during encoding and recognition was examined by entering individual activation contrasts from the control condition in all participants into one-sample t-tests (reported at $p < .05$ family wise error (FWE) corrected for multiple comparisons). Activation differences between responders and non-responders in the control and fatigue conditions, and the interaction between cortisol response group and fatigue condition were investigated at the whole brain level, as well as within hippocampal, amygdala and PFC regions using second level two (responders vs. non-responders) by two (control vs.

fatigue condition) Full Factorial models, for encoding and recognition separately. Whole brain effects were examined at p (FWE) < .05 and at the uncorrected level p < .001 (with a cluster size threshold > 10 voxels, masked inclusively at p < .05 to restrict the search for effects to those voxels that showed a main effect of task). ROI analyses were conducted using the MarsBaR toolbox for SPM (Brett et al., 2002). ROIs were defined using AAL areas (Tzourio-Mazoyer et al., 2002) including the bilateral hippocampus, amygdala, superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus and anterior cingulate cortex (with PFC areas selected based on primary encoding and recognition activation found in a meta-analysis by Spaniol et al., 2009), with reported effects significant after correction for multiple comparisons (p < .05).

Behavioural and salivary cortisol statistics

Behavioural and salivary cortisol analyses were carried out using PASWstatistics (version 18.0). We conducted a k-means cluster analysis based on cortisol response (AUC_I values) to the fatigue condition to identify responder and non-responder groups (in line with previous studies e.g., Khalili-Mahani et al., 2010). To gain insight into the change in cortisol levels throughout the test sessions in each group, cortisol values at each time point were also examined. One-way analysis of variance (ANOVA) was used to compare responders and non-responders on the neuropsychological tests and past-week fatigue VAS. The effects of cortisol response group and induced fatigue condition on the POMS subscales and encoding and recognition task performance were investigated using repeated measures ANOVA.

RESULTS

The cluster analysis identified 15 responders and 12 non-responders. As groups were created post-hoc, the order of fatigue condition administration was not balanced and was therefore included as a covariate in all analyses (5 non-responders and 9 responders started in the control condition). Furthermore, although mean age did not differ significantly between groups (non-responders mean age = 43.3, SD = 13.4, range = 27 – 61; responders mean age = 45.8, SD = 12.6, range = 25 – 60), age was also included as a covariate (continuous variable) in all analyses in light of the relatively wide age range of our sample. The groups were also unbalanced with regard to time of day of test administration (non-responders: 2 x 0900 h, 4 x 1100 h, 6 x 1300 h; responders: 5 x 0900 h, 7 x 1100 h, 2 x 1300 h). Time of day is an important factor in cortisol measurement due to diurnal fluctuations in cortisol levels. However, since we calculated AUC_I values instead of the total cortisol output, baseline differences in cortisol were unlikely to have an effect. Indeed, Kudielka et al. (2004) showed that the stress-induced salivary cortisol increase from baseline does not differ in the morning and afternoon (but see Dickerson and Kemeny, 2004a). Nevertheless, we conducted a repeated measures ANOVA that confirmed that AUC_I values did not differ significantly between the different test times, and time of day did not interact with fatigue condition.

Salivary cortisol

Cortisol levels (Figure 1) were significantly higher in responders than non-responders in the fatigue condition at time 1 ($F(1, 23) = 13.72$, $p = .001$), time 2 ($F(1, 23) = 4.98$, $p = .036$) and time 3 ($F(1, 23) = 5.83$, $p = .024$). Furthermore, non-responders showed a significant decrease in cortisol levels from time 0 to time 1 in the control ($t(11) = 4.14$, $p = .002$) and the fatigue condition ($t(11) = 6.87$, $p < .001$). Responders, on the other hand, showed an increase in cortisol levels from time 0 to time 1 in the fatigue condition ($t(14) = 2.75$, $p = .016$). However, inspection of fatigue condition AUC_I values (see Supplementary material Figure S1, pp100) revealed that our 'responder' group can be more accurately described as consisting of participants showing an increase in cortisol levels as well as participants showing little change in cortisol level, whereas the 'non-responder' group consists of participants showing a decrease in cortisol levels.

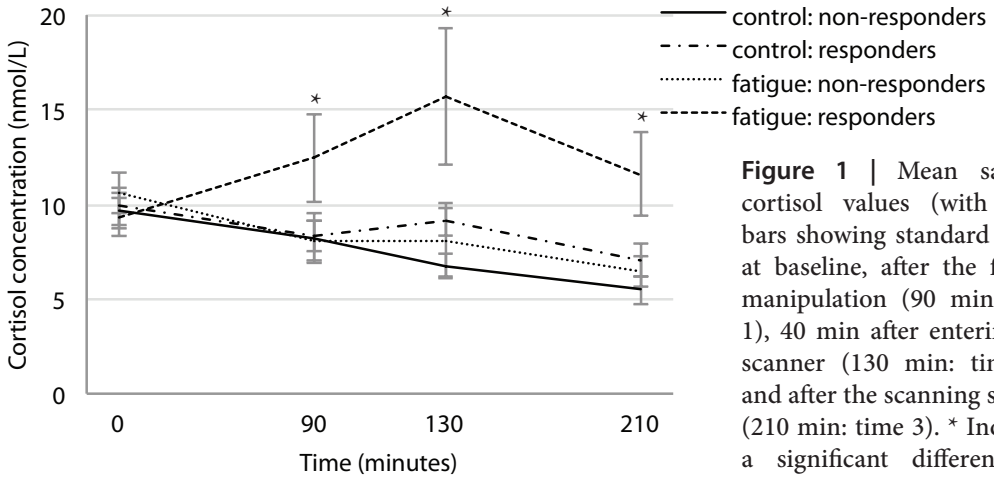


Figure 1 | Mean salivary cortisol values (with error bars showing standard error) at baseline, after the fatigue manipulation (90 min: time 1), 40 min after entering the scanner (130 min: time 2), and after the scanning session (210 min: time 3). * Indicates a significant difference in cortisol concentration.

Neuropsychological test performance

Scores did not differ significantly between responders and non-responders on any of the tests (Table 1).

Table 1 | Neuropsychological test scores

	Non-responders			Responders		
	Mean	SD	Range	Mean	SD	Range
WLT immediate free recall	11.7	2.4	7 – 15	11.4	2.1	7 – 15
WLT delayed free recall	10.3	3.4	5 – 15	10.3	2.5	6 – 14
WLT delayed cued recall	14.3	1.1	12 – 15	14.4	0.9	12 – 15
Digit span	17.8	3.8	13 – 24	16.2	3.4	10 – 24
LDST	55.8	7.2	47 – 70	53.7	8.4	37 – 66
Letter fluency	16.0	3.6	11 – 23	15.0	5.1	8 – 22
National adult reading test	84.5	8.4	65 – 96	85.5	5.5	73 – 93

Subjective ratings

Analysis of the POMS subscales (Table 2) showed a near-significant main effect of cortisol response group ($F(1, 23) = 4.15$, $p = .053$) on the fatigue subscale. Follow-up tests showed higher fatigue ratings in responders than non-responders in the fatigue condition following the manipulation (time 1: $F(1, 23) = 5.70$, $p = .026$) and persisting till after the scan session (time 3: $F(1, 23) = 4.67$, $p = .041$). A main effect of cortisol response group was found on the vigour subscale ($F(1, 23) = 9.55$, $p = .005$), indicating lower vigour in responders than non-responders. A follow-up test confirmed that vigour levels also differed between groups at baseline (time 0: $F(1, 23) = 5.33$, $p = .030$).

A main effect of fatigue condition was found on the tension subscale ($F(1, 23) = 10.07$, $p = .004$), along with a (near) significant interaction between fatigue condition and time ($F(2, 46) = 3.26$, $p = .051$). Follow-up tests showed that tension ratings were higher in all participants following the fatigue compared to the control manipulation (time 1) ($F(1, 23) = 4.85$, $p = .038$).

Ratings on the past-week VAS questions showed that responders felt that fatigue interfered more with their ability to plan activities ($F(2, 26) = 5.75$, $p = .020$; responders mean = 29.8, SD = 20.2; non-responders mean = 14.8, SD = 7.2), think clearly ($F(2, 26) = 5.79$, $p = .024$; responders mean = 33.7, SD = 20.2; non-responders mean = 18.3, SD = 12.5) and (a trend to) think quickly ($F(2, 26) = 3.76$, $p = .064$; responders mean = 39.9, SD = 24.0; non-responders mean = 23.5, SD = 16.6) than non-responders.

Table 2 | Mean subjective ratings (with standard deviation)

		Non-responders		Responders	
	Time	Control	Fatigue	Control	Fatigue
Fatigue	0	16 (12)	21 (16)	25 (11)	26 (9)
	1*	21 (12)	25 (16)	27 (12)	38 (14)
	3*	27 (16)	34 (13)	32 (16)	47 (18)
Vigour	0*	88 (10)	83 (14)	77 (11)	76 (13)
	1*	81 (12)	78 (15)	64 (15)	63 (14)
	3*	74 (18)	72 (13)	64 (20)	55 (14)
Tension	0	14 (10)	16 (19)	18 (9)	20 (12)
	1	12 (11)	18 (12)	15 (8)	24 (11)
	3	14 (12)	15 (12)	16 (10)	17 (6)

* Significant differences between responders and non-responders. Time 0 = baseline, time 1 = following the fatigue manipulation, time 3 = at the completion of the scan session.

Encoding and recognition task performance

No significant differences in accuracy (Table 3) or RT were found between responders (control mean = 1152 ms, SD = 158; fatigue mean = 1148 ms, SD = 138) and non-responders during encoding (control mean = 1160 ms, SD = 103; fatigue mean = 1152

ms, SD = 141). RTs also did not differ between responders (control mean = 1343 ms, SD = 185; fatigue mean = 1296 ms, SD = 218) and non-responders (control mean = 1383 ms, SD = 139; fatigue mean = 1383 ms, SD = 166) during recognition. Recognition task accuracy differences were found with regard to high confidence corrected recognition scores: scores were higher in non-responders than responders ($F(1, 23) = 4.43$, $p = .046$), although no interaction was found with fatigue condition.

Table 3 | Mean accuracy (with standard deviation) on the encoding and recognition tasks

	Non-responders		Responders	
Judgment	Control	Fatigue	Control	Fatigue
Encoding task				
Accuracy	94 (3)	93 (4)	93 (5)	94 (4)
Recognition task				
High confidence hits	58 (20)	62 (11)	52 (21)	54 (20)
High confidence corrected recognition*	47 (16)	51 (11)	39 (14)	38 (13)
Low confidence hits	14 (11)	14 (8)	19 (11)	16 (8)
Misses	27 (12)	22 (13)	29 (15)	29 (15)
High confidence correct rejections	48 (27)	44 (24)	39 (16)	40 (18)
Low confidence correct rejections	29 (22)	30 (15)	32 (12)	30 (14)
High confidence false alarms	11 (8)	11 (9)	13 (12)	16 (14)
Low confidence false alarms	10 (9)	13 (10)	15 (9)	13 (10)

* Significant differences between responders and non-responders

fMRI results

Task-related activation in the control condition related to encoding and recognition, regardless of cortisol response, is provided in Supplementary material Table S1 (pp99). No whole-brain activation differences were found between responders and non-responders in the control condition during encoding or recognition. In the fatigue condition, activation was significantly greater in non-responders than responders during both encoding and recognition (Table 4). Encoding activation was greater in non-responders in the right VLPFC and temporo-parietal junction, and the bilateral superior parietal cortex and putamen. Recognition activation was greater in non-responders in the right DMPFC, bilateral VLPFC, left posterior temporal cortex and right superior and bilateral inferior parietal cortex, left caudate and putamen, and right thalamus. An interaction between the effect of cortisol response group and fatigue condition on encoding activation was found at the whole brain level in the right hippocampus that neared significance at $p(FWE) < .05$ (coordinates x = 27, y = -12, z = -21; t-value = 5.07; cluster size = 89, $p(FWE) = .052$) (Figure 2a), whereas no interaction effect was evident in relation to recognition activation.

Table 4 | Activation differences between responders and non-responders in the fatigue condition during encoding and recognition

Region		BA	MNI coordinates			t-value	Cluster size (voxels)
			X	Y	Z		
Encoding task: Non-responders > Responders							
Ventrolateral PFC	R	48/47/45	39	30	9	3.94	10
Temporo-parietal	R	48	51	-24	24	5.57	69*
Superior parietal	L	7	-6	-57	60	3.62	11
	R	7	12	-60	66	4.12	117*
Putamen	L	-	-18	9	-9	3.96	12
	R	-	21	15	-3	3.74	31
Recognition task: Non-responders > Responders							
Dorsomedial PFC	L	8	-6	27	54	3.87	41
	L	8	-21	6	54	3.48	14
Ventrolateral PFC	L	45	-42	33	27	4.31	46
	R	48	51	21	18	3.81	43
Posterior temporal	L	21	-42	-39	6	4.27	23
	L	*37*	*-45*	*-51*	*3*	*3.84*	
Superior parietal	R	5	15	-57	54	3.97	87
	R	*7*	*15*	*-66*	*45*	*3.57*	
Inferior parietal	L	40	-48	-45	45	3.80	37
	R	40	39	-45	39	3.51	12
Caudate	L	-	-12	9	6	4.31	46
Putamen	*L*	-	*-21*	*18*	*-3*	*3.54*	
Thalamus	R	-	12	-18	12	3.91	23

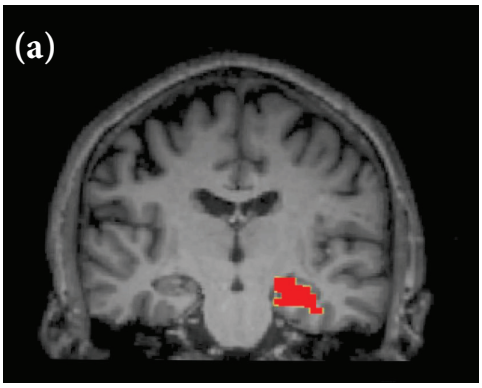
Effects are significant at p(uncorrected) < .001, with * indicating clusters significant at p(FWE) < .05. Italics indicate multiple peak activation voxels within an activation cluster.

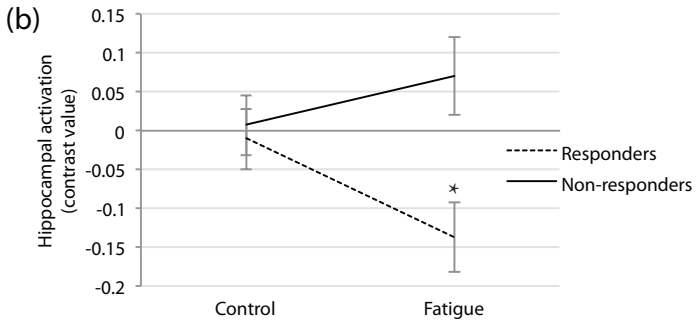
ROI analysis confirmed a significant interaction during encoding within the right hippocampal ROI (t-value = 2.95, p = .034), but did not reveal an interaction effect in any of the other ROIs during either encoding or recognition. The interaction in the right hippocampal ROI reflected reduced encoding activation in the fatigue compared to the control condition in responders (t-value = 1.99, p = .027), but somewhat of an increase in activation in non-responders (t-value = 1.56, p = .062) (Figure 2b). Furthermore, activation in the right hippocampal ROI was greater in non-responders than responders in the fatigue condition (t-value = 3.12, p = .002), but did not differ in the control condition. Greater activation in non-responders than responders in the fatigue condition was also evident in the right inferior frontal gyrus ROI during both encoding (t-value = 3.04, p =

.027) and recognition (t-value = 3.07, p = .024), and in the left superior frontal gyrus ROI during recognition (t-value = 3.12, p = .002).

fMRI post-hoc correlations

In ROIs showing a significant interaction between cortisol response group and fatigue condition (the right hippocampal ROI), we also examined the relationship between activation differences (control compared to fatigue condition) and subjective fatigue ratings (the change from time 0 to time 1 in the control compared to fatigue condition) and task performance (high confidence corrected recognition scores in the control compared to fatigue condition) using a simple regression analysis in SPM8. In the responder group, a significant negative relationship was found between encoding activation in the right hippocampal ROI and fatigue ratings (t-value = 2.11, p = .028), indicating that a greater decrease in activation (in the fatigue compared to the control condition) was associated with a greater increase in fatigue ratings (Figure 2c).





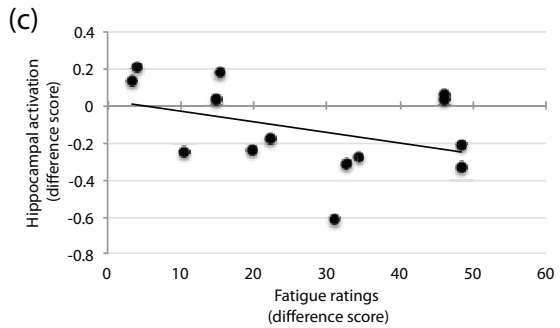


Figure 2 |

a) The interaction effect in the right hippocampus.

b) Right hippocampal activation in responders and non-responders in the control and fatigue conditions (* indicates a significant difference between the control and fatigue condition).

c) The negative relationship between hippocampal activation difference scores (fatigue minus control condition) and subjective fatigue rating difference scores in responders.

DISCUSSION

We investigated the relationship between cortisol and cognitive fatigue induced by the sustained performance of cognitively demanding tasks. The fatigue condition resulted in higher subjective fatigue ratings, indicating that a state of increased fatigue was successfully induced compared to the control condition. Furthermore, an accompanying increase in feelings of tension was found, providing some indication of the elicitation of a stress response in the fatigue condition. Analysis of salivary cortisol levels demonstrated that the fatigue manipulation indeed elicited a stress response, indexed by elevated cortisol levels, in a group of 15 responders out of the total 27 participants. Studies using psychological stress paradigms have similarly reported the categorisation of around 50% of participants as responders (Dedovic et al., 2009a). Inspection of cortisol levels throughout the test session showed that cortisol levels increased further after participants entered the scanner, indicating that the scanner itself may also have contributed to the stress response (Muehlhan et al., 2011).

Cortisol responders and non-responders were well matched with regard to demographic characteristics including gender, education level and occupation as a result of the homogeneous study population of male school teachers. Moreover, the two groups did not differ significantly with respect to baseline cortisol levels, subjective fatigue and tension ratings or neuropsychological task performance. However, subjective vigour ratings revealed lower feelings of vigour in responders than in non-responders at baseline. Furthermore, responders indicated that they felt that fatigue interfered more with their ability to plan activities and think clearly* during the last week than non-responders. Therefore, it is possible that more long-term differences in fatigue and vigour levels may exist between our responder and non-responder groups. This possibility should be investigated further with the inclusion of more comprehensive long-term fatigue measures, along with measures of chronic HPA axis function.

Differences between cortisol responders and non-responders were found in relation to subjective fatigue ratings, memory performance and memory-related brain activation. Responders showed higher fatigue ratings than non-responders in the fatigue condition, supporting an association between cortisol and fatigue. This finding indicates that feelings of fatigue arising from the sustained performance of cognitively demanding tasks may be exacerbated when accompanied by a physiological stress response. In line with our hypothesis, we suggest that higher fatigue levels in responders may reflect greater resource exhaustion in this group following an initially greater increase in resource mobilisation in relation to the cortisol response. However, future studies should collect more frequent fatigue and cortisol measurements in order to obtain a more detailed picture of the relationship between these factors over time. Furthermore, more research is needed to elucidate the complex relationship between acute-immediate, acute-prolonged and chronic cortisol levels and fatigue.

Responders showed poorer recognition accuracy than non-responders, however, this difference was evident in both the control and fatigue condition. Hence, increased cortisol levels in responders in the fatigue condition were not associated with greater recognition impairment. This difference therefore reflects baseline differences in recognition accuracy

between the responder and non-responders groups, perhaps indicating that responders found the memory tasks more difficult and, consequentially, more stressful than non-responders.

Reduced brain activation was evident in responders during encoding and recognition in the fatigue condition in comparison to non-responders, in line with the suggestion that responders are primarily characterised by greater whole-brain activation decreases (Pruessner et al., 2008). The investigation of the interaction between cortisol response and fatigue condition revealed a significant interaction in the right hippocampus during encoding. This interaction reflected significantly decreased activation in the fatigue compared to the control condition in responders, but somewhat of an increase in activation in non-responders. This difference in hippocampal activation between responders and non-responders may be associated with differences in encoding efficiency. Indeed, the hippocampus has been consistently implicated in memory encoding. However, in line with previous studies (Spaniol et al., 2009), more robust task-related hippocampal activation during verbal memory encoding was evident in the left rather than the right hippocampus. Furthermore, the change in hippocampal activation correlated significantly with subjective fatigue levels, rather than subsequent recognition performance. Therefore, we suggest that activation differences in the right hippocampus may more likely reflect the involvement of this area in differences in arousal/fatigue state (e.g., via the nucleus incertus: Ryan et al., 2011) between responders and non-responders than cortisol-induced blockade of memory encoding processes.

Nevertheless, our findings are congruous with the suggestion that the hippocampus plays a central role with regard to activation differences between responders and non-responders (Dedovic et al., 2009b; Khalili-Mahani et al., 2010; Pruessner et al., 2008). Moreover, our findings extend current knowledge by demonstrating that the right hippocampus may also play a central role in the relationship between short-term changes in cortisol and fatigue levels. However, we did not find support for the suggestion that the pre-stress activation state of the hippocampus is involved in determining individual differences in the cortisol response (Khalili-Mahani et al., 2010), as hippocampal activation did not differ significantly between responders and non-responders in the control condition. The primary involvement of the hippocampus in differences between responders and non-responders is unsurprising given the high concentration of cortisol receptors in this area. Accordingly, it has been suggested that the hippocampus may play a critical role in "processing psychological conditions that trigger the HPA axis response" (Herman and Cullinan, 1997). It is therefore possible that activation changes in the right hippocampus may be central to both the elicitation of short-term changes in HPA axis function and associated fatigue levels as well as in the etiology of HPA axis dysfunction and chronic fatigue.

With regard to the comparison of responders to non-responders in general, we note that due to the high inter-individual variability in the cortisol response, responder and non-responder groups represented a considerable range of cortisol responses. Hence, fMRI studies with larger samples sizes are needed to allow the creation of more homogeneous cortisol response groups to better investigate group differences.

In conclusion, we demonstrate that individual differences in the cortisol response elicited

by an induced fatigue paradigm were associated with significant differences in the effect of this paradigm on subjective fatigue ratings and brain activation. Findings provide insight into individual differences in the severity of induced fatigue effects and indicate that individuals who react with a physiological stress response to, for example, a demanding workday may suffer exacerbated fatigue-related complaints. These results are particularly relevant to population groups engaged in professions with high cognitive demands, such as the school teachers examined in the present study. However, due to their busy work schedule, participants representing such population groups are also particularly difficult to recruit, especially for fMRI studies requiring a substantial time commitment. Hence, our study is somewhat limited by a small sample size. Nevertheless, studies such as ours in working professionals are important to our understanding of factors affecting workplace performance and work-related fatigue.

REFERENCES

Bangasser, D.A., Shors, T.J., 2010. Critical brain circuits at the intersection between stress and learning. Neurosci Biobehav Rev 34, 1223-33.

Beck, K.D., Luine, V.N., 2010. Evidence for sex-specific shifting of neural processes underlying learning and memory following stress. Physiol Behav 99, 204-11.

Boksem, M.A., Tops, M., 2008. Mental fatigue: costs and benefits. Brain Res Brain Res Rev 59, 125-39.

Boksem, M.A., Meijman, T.F., Lorist M.M., 2006. Mental fatigue, motivation and action monitoring. Biol Psychol 72, 123-32.

Bohnen, N., Houx, P., Nicolson, N., Jolles, J., 1990. Cortisol reactivity and cognitive performance in a continuous mental task paradigm. Biol Psychol 31, 107-16.

Brett, M., Anton, J., Valabreque, R., Poline, J., 2002. Region of interest analysis using an SPM toolbox [abstract] Presented at the 8th International Conference on Functional Mapping of the Human Brain, Sendai, Japan. Neuroimage 16: Available on CD-ROM.

Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol Psychol 80. 265-78.

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., Pruessner, J.C., 2009a. The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. Neuroimage 47, 864-71.

Dedovic, K., Rexroth, M., Wolff, E., Duchesne, A., Scherling, C., Beaudry, T., et al., 2009b. Neural correlates of processing stressful information: an event-related fMRI study. Brain Res 1293, 49-60.

DeLuca, J., 2005. Fatigue, Cognition and Mental Effort. In: DeLuca J. (Ed.), Fatigue as a window to the brain. Cambridge, Mass. ; London: MIT Press, pp. 37-58.

de Quervain, D.J., Roozendaal, B., Nitsch, R.M., McGaugh, J.L., Hock, C., 2000. Acute cortisone administration impairs retrieval of long-term declarative memory in humans. Nat Neurosci 3, 313-4.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull 130, 355-91.

Dijkers, M.P., Bushnik, T., 2008. Assessing fatigue after traumatic brain injury: an evaluation of the Barroso Fatigue Scale. J Head Trauma Rehab 23, 3-16.

Gold, P.W., Drevets, W.C., Charney, D.S., 2002. New insights into the role of cortisol and the

glucocorticoid receptor in severe depression. Biol Psychiat 52, 381-5.

Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamic-pituitary-adrenocortical axis. Trends Neurosci 20, 78-84.

Jerjes, W.K., Cleare, A.J., Wessely, S., Wood, P.J., Taylor, N.F., 2005. Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome. J Affect Dis 87, 299-304.

Kato, Y., Endo, H., Kizuka, T., 2009. Mental fatigue and impaired response processes: event-related brain potentials in a Go/NoGo task. Int J Psychophysiol 72, 204-11.

Khalili-Mahani, N., Dedovic, K., Engert, V., Pruessner, M., Pruessner, J.C., 2010. Hippocampal activation during a cognitive task is associated with subsequent neuroendocrine and cognitive responses to psychological stress. Hippocampus 20, 323-34.

Kudielka, B.M., Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2004. Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. Psychoneuroendocrinology 29, 983-92.

Kukolja, J., Thiel, C.M., Wolf, O.T., Fink, G.R., 2008. Increased cortisol levels in cognitively challenging situations are beneficial in young but not older subjects. Psychopharmacology 201, 293-304.

Kumari, M., Badrick, E., Chandola, T., Adam, E.K., Stafford, M., Marmot, M.G., et al., 2009. Cortisol secretion and fatigue: associations in a community based cohort. Psychoneuroendocrinology 34, 1476-85.

Lezak, M.D., Howieson, D.B., Loring, D.W., 2004. Neuropsychological Assessment. 4th ed. New York: Oxford University Press, Inc.

Lorist, M.M., 2008. Impact of top-down control during mental fatigue. Brain Res 1232, 113-123.

Lorist, M.M., Boksem, M.A., Ridderinkhof, K.R., 2005. Impaired cognitive control and reduced cingulate activity during mental fatigue. Brain Res Cogn Brain Res 24, 199-205.

Lupien, S.J., Maheu, F., Tu, M., Fiocco, A., Schramek, T.E., 2007. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain Cognition 65, 209-237.

McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: Central role of the brain. Physiol Rev 87, 873-904.

Muehlhan, M., Lueken, U., Wittchen, H.U., Kirschbaum, C., 2011. The scanner as a stressor: Evidence from subjective and neuroendocrine stress parameters in the time course of a functional magnetic resonance imaging session. Int J Psychophysiol 79, 118-126.

Nater, U.M., Youngblood, L.S., Jones, J.F., Unger, E.R., Miller, A.H., Reeves, W.C., Heim, C., 2008. Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. Psychosom Med 70, 298-305.

Nelson, H.E., 1991. National adult reading test (NART), 2nd ed ed. NFER-NELSON, Windsor.

Nicolson, N., van Diest, R., 2000. Salivary cortisol patterns in vital exhaustion. J Psychosom Res 49, 335-342.

O'Connor, P.J., 2006. Mental energy: Assessing the mood dimension. Nutr Rev 64, S7-9.

Oei, N.Y., Elzinga, B.M., Wolf, O.T., de Ruiter, M.B., Damoiseaux, J.S., Kuijer, J.P., Veltman, D.J., Scheltens, P., Rombouts, S.A., 2007. Glucocorticoids Decrease Hippocampal and Prefrontal Activation during Declarative Memory Retrieval in Young Men. Brain Imaging Behav 1, 31-41.

Pruessner, J.C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., Renwick,

R., Dagher, A., Meaney, M.J., Lupien, S., 2008. Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. Biol Psychiat 63, 234-240.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916-931.

Reuter, M., 2002. Impact of cortisol on emotions under stress and nonstress conditions: a pharmacopsychological approach. Neuropsychobiology 46, 41-48.

Rimes, K.A., Chalder, T., 2005. Treatments for chronic fatigue syndrome. Occup Med (Oxf) 55, 32-39.

Ryan, P.J., Ma, S., Olucha-Bordonau, F.E., Gundlach, A.L., 2011. Nucleus incertus--an emerging modulatory role in arousal, stress and memory. Neurosci Biobehav Rev 35, 1326-1341.

Silverman, M.N., Heim, C.M., Nater, U.M., Marques, A.H., Sternberg, E.M., 2010. Neuroendocrine and immune contributors to fatigue. PM&R 2, 338-346.

Spaniol, J., Davidson, P.S.R., Kim, A.S.N., Han, H., Moscovitch, M., Grady, C.L., 2009. Event-related fMRI studies of episodic encoding and retrieval: Meta-analyses using activation likelihood estimation. Neuropsychologia 47, 1765-1779.

Tops, M., van Peer, J.M., Wijers, A.A., Korf, J., 2006. Acute cortisol administration reduces subjective fatigue in healthy women. Psychophysiology 43, 653-656.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15, 273-289.

Van der Elst, W., van Boxtel, M.P., van Breukelen, G.J., Jolles, J., 2005. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. J Int Neuropsychol Soc 11, 290-302.

van der Elst, W., van Boxtel, M.P., van Breukelen, G.J., Jolles, J., 2006a. The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. J Clin Exp Neuropsychol 28, 998-1009.

Van der Elst, W., Van Boxtel, M.P., Van Breukelen, G.J., Jolles, J., 2006b. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. J Int Neuropsychol Soc 12, 80-89.

van der Linden, D., Frese, M., Meijman, T.F., 2003. Mental fatigue and the control of cognitive processes: effects on perseveration and planning. Acta Psychol 113, 45-65.

van Stegeren, A.H., 2009. Imaging Stress Effects on Memory: A Review of Neuroimaging Studies. Can J Psychiat 54, 16-27.

Wald, F.D.M., Mellenbergh, G.J., 1990. The short version of the Dutch version of the Profile of Mood States. Nederlands Tijdschrift voor de Psychologie 45, 86-90.

Wang, J., Rao, H., Wetmore, G.S., Furlan, P.M., Korczykowski, M., Dinges, D.F., Detre, J.A., 2005. Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. Proc Natl Acad Sci USA 102, 17804-17809.

SUPPLEMENTARY MATERIAL

Table S1 | Task-related encoding and recognition activation in all participants in the control condition

Region		BA	MNI coordinates			t-value	Cluster size (voxels)
			X	Y	Z		
Encoding task							
Dorsomedial PFC	L/R	32	0	12	51	7.39	91
	L/R	*6*	*-6*	*3*	*63*	*7.12*	
	L/R	*32*	*-6*	*15*	*45*	*6.95*	
Precentral gyrus	R	6	33	-3	48	7.23	77
Ventrolateral PFC	L	44	-51	6	30	10.60	618
	L	*45*	*-45*	*27*	*18*	*9.06*	
Insula	R	47	33	24	-3	6.39	17
Inferior parietal	L	40	-33	-51	48	14.87	642
	L	*2*	*-48*	*-36*	*51*	*9.41*	
	L	*7*	*-24*	*-66*	*39*	*8.72*	
	R	7	30	-51	48	9.21	142
	R	*7*	*24*	*-63*	*57*	*7.48*	
Occipital	L	17	-27	-96	6	12.50	902
	L	*17*	*-15*	*-99*	*-6*	*12.14*	
	L	*18*	*-24*	*-90*	*-3*	*11.34*	
	R	19	30	-69	33	6.19	5
Hippocampus	L	37	-27	-30	-6	7.58	49
Thalamus	*L*	-	*-18*	*-27*	*12*	*6.14*	
Recognition task							
Anterior cingulate	L	32	-6	12	48	6.67	38
Orbital frontal	R	47	30	27	-9	7.12	25
Insula	L	47	-30	24	0	6.06	5
Fusiform	L	19	-39	-75	-12	7.49	31
Inferior parietal	L	40	-33	-51	45	8.91	80
Occipital	L	18	-27	-99	3	11.38	120
	L	*17*	*-15*	*-99*	*-6*	*11.03*	
	L	*18*	*-24*	*-96*	*-9*	*9.09*	
	R	17	12	-90	-3	7.05	5
	R	18	15	-87	-6	6.17	2
	R	18	30	-90	12	6.04	3

Effects are significant at p(FWE) < .05. Italics indicate multiple peak activation voxels within an activation cluster.

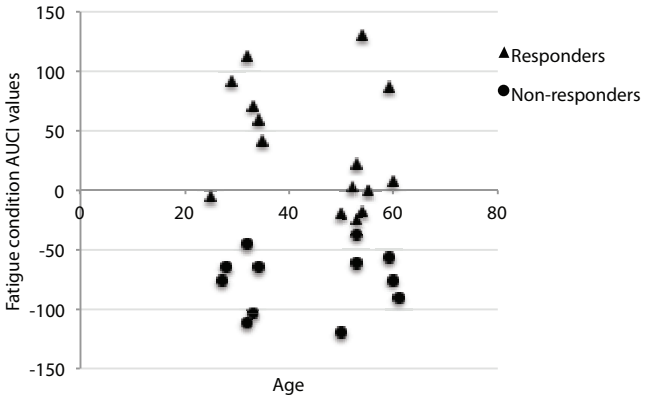


Figure S1 | The distribution of cortisol AUC_I values in the fatigue condition as a function of age in the responder and non-responder groups.