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Glucocorticoid receptor number predicts increase in amygdala activity after severe stress

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Summary
Introduction: Individuals who are exposed to a traumatic event are at increased risk of developing psychiatric disorders such as posttraumatic stress disorder (PTSD). Studies have shown that increased amygdala activity is frequently found in patients with PTSD. In addition, pre-trauma glucocorticoid receptor (GR) number in peripheral blood mononuclear cells (PBMCs) has been found to be a significant predictor for the development of PTSD symptoms. Research in rodents has shown that the response of basolateral amygdala neurons to corticosterone is mediated by GR. However, to the best of our knowledge, no previous study has investigated GR number in PBMCs and amygdala function in humans.
Methods: To investigate whether peripheral GR number is related to amygdala functioning, we assessed GR number in PBMCs of healthy soldiers before their deployment to Afghanistan. Amygdala functioning was assessed with fMRI before and after deployment.
Results: We found that pre-deployment GR number was significantly negatively correlated to pre-deployment amygdala activity. More importantly, pre-deployment GR number predicted the increase in amygdala activity by deployment.
Discussion: Our results demonstrate that peripheral GR number is associated with amygdala functioning and predicts the increase in amygdala activity following military deployment in

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healthy individuals who did not develop PTSD. It is uncertain how this relationship is mediated mechanistically, but future studies should examine the relation of GR and amygdala activity to determine whether this is part of a common pathway leading to increased vulnerability to stress-related disorders.

1. Introduction

Individuals who are exposed to a traumatic event (such as natural disasters, terrorism, assault, or military combat) are at risk of developing psychiatric disorders such as posttraumatic stress disorder (PTSD). In the last decades knowledge of the lasting consequences of traumatic stress on the neurobiology of individuals has increased rapidly. Functional neuroimaging studies have revealed that patients with PTSD show exaggerated amygdala responses and deficient prefrontal cortex function, in particular in the anterior cingulate cortex and ventromedial prefrontal cortex (Rauch et al., 2000; Shin et al., 2001, 2005). These neural alterations in PTSD probably result from both stress exposure and stress vulnerability (Admon et al., 2009; Shin et al., 2009). In a recent study, we investigated soldiers before and after their first deployment to a combat zone (van Wingen et al., 2011). Our results demonstrated that combat stress increases amygdala and insula reactivity to biologically salient stimuli, and that impaired prefrontal cortex—amygdala coupling was associated with perceived threat. Follow-up showed that amygdala responsivity normalizes within 1.5 years after deployment in resilient individuals that do not develop PTSD, but that perceived threat-dependent changes in amygdala coupling with the dorsal anterior cingulate cortex persist (van Wingen et al., 2012).

In addition to these neurobiological changes, numerous studies have also shown that PTSD is associated with increased sensitivity of the hypothalamic—pituitary—adrenal axis (HPA-axis) to negative feedback by glucocorticoids (GCs) (Yehuda et al., 1995, 2002; Golier et al., 2006; de Kloet et al., 2006, 2007a,b). Recently, we reported that the pre-trauma glucocorticoid receptor number in peripheral blood mononuclear cells (PBMCs) was higher within soldiers who developed a high level of PTSD symptoms after deployment to Afghanistan compared to matched controls who did not develop PTSD symptoms (van Zuiden et al., 2011, 2012). Although the peripheral GR pathway is often considered to be an accessible model for GR signaling in the brain, it remains unclear if and how peripheral GR functioning is related to CNS activity. Preclinical research has shown that the response of basolateral amygdala neurons to corticosterone is mediated by GR (Karst et al., 2010; Arnett et al., 2011; Groeneweg et al., 2011). Whether GR number in PBMCs is a reliable reflection of GR number in the brain has not been studied extensively. Preclinical studies have shown that the affinity and specificity for GCs of neuronal and lymphoid GRs are similar (Lowy, 1989). In addition, GRs in both brain and peripheral immune tissues are downregulated after chronic corticosterone administration (Spencer et al., 1991). However, to our knowledge, no previous study has investigated the relation between peripheral GR and amygdala function in humans. We hypothesized that GR number in PBMCs would be related to amygdala functioning in healthy individuals. In addition, we hypothesized that GR number in PBMCs would be associated with the amygdala response in reaction to severe stress.

To investigate whether peripheral GR is related to amygdala functioning, we assessed GR number in PBMCs in soldiers before deployment to Afghanistan. Amygdala functioning was assessed with functional magnetic resonance imaging (fMRI) in the same group of soldiers before and 1.5 months after deployment.

2. Methods

2.1. Participants

Participants included in this study consisted of twenty-four healthy soldiers who were deployed for 4 months to Afghanistan as part of the NATO International Security Assistance Force (ISAF) peacekeeping operation. They were recruited from a larger prospective study on the development of stress-related disorders following military deployment in the Dutch armed forces. None of the participants had been deployed previously. Duties during deployment included combat patrols, clearing or searching homes and buildings, participation in demining operations, and transportation across enemy territory. They were exposed to typical war-zone stressors such as enemy fire, armed combat, and seeing seriously injured fellow soldiers and civilians (including women and children). The study was in accordance with the declaration of Helsinki and institutional guidelines of the local ethics committee (CMO Arnhem-Nijmegen, the Netherlands and the Institutional Review Board of the University Medical Center Utrecht, the Netherlands), and all participants provided written informed consent after written and oral description of the study.

2.2. Procedure

Participants were investigated several weeks prior to deployment to Afghanistan. A venous blood sample was drawn for assessment of the number of GR binding sites in PBMCs. Participants filled in a number of paper-and-pencil questionnaires. Since GR is associated with glucose regulation, and polymorphisms of the GR gene are associated with metabolic parameters and body composition (van Rossum and Lamberts, 2004), we also measured the body-mass index (BMI) prior to deployment. BMI was calculated from the self-reported weight and length of the participants. In addition, all participants received an fMRI scan before and 1.5 months after deployment.

2.3. Questionnaires

A previously validated Dutch questionnaire, the Self-rating Inventory for PTSD, was used to assess self-reported PTSD

symptoms (Hovens et al., 1994). We included all healthy soldiers who were deployed for 4 months to Afghanistan. As it is quite plausible that GR concentrations and amygdala activity would be altered in those individuals who develop PTSD both prior to deployment and after, one participant who scored above the clinical threshold for PTSD symptoms after deployment was therefore excluded from the analyses. Exposure to potential traumatic experiences before the age of 18 was assessed using the Dutch version of the short form self-report version of the Early Trauma Inventory (Bremner et al., 2007; Rademaker et al., 2008). This questionnaire consists of 27 dichotomous items. The total score represents the number of experienced events. To quantify stress exposure, we measured combat exposure during deployment using the combat experience scale of the Deployment Risk and Resilience Inventory (Vogt et al., 2008).

2.4. Cortisol

Plasma cortisol levels were measured by electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics, Mannheim, Germany). Lower limit of detection was 3 nmol/l; interassay variation was <3%; and reference values (0700–1000 h) were 170–540 nmol/l.

2.5. GR binding sites in PBMCs

For determination of the capacity of PBMCs to bind glucocorticoids a validated whole cell single point binding assay was used as described previously. This method provides a reliable estimate of B_{max} as determined using a classical binding assay with 3–200 nM ^{3}H-dexamethasone ($r^2 = 0.92$) (van Zuiden et al., 2009, 2011). Briefly, PBMCs were isolated from whole blood using Ficoll-Paque (Pharmacia, Uppsala, Sweden) and 10^7 cells were frozen in DMSO. After thawing and 60 min equilibration in culture medium, cells were washed twice, resuspended in assay buffer (RPMI-1640 with 5% FCS) and incubated in duplicate with 100 nM ^{3}H-dexamethasone (Amersham, Buckinghamshire, UK) in the presence or absence of excess unlabeled dexamethasone (Sigma–Aldrich, Steinheim, Germany) for 1 h at 37 °C. Cell bound radioactivity was quantified by liquid scintillation analysis.

2.6. MRI data acquisition

MR data were acquired with a 1.5 T Siemens (Erlangen, Germany) Avanto MR scanner, equipped with a standard head coil. T2*-weighted BOLD images were acquired using EPI with an echo time of 35 ms to reduce signal dropout in the medial temporal lobes. Each image volume consisted of 32 axial slices (3.5 mm, 0.35 mm slice-gap, TR = 2.340 s, 64 × 64 matrix, FOV = 212 mm, FA = 90°). In addition, a high-resolution T1-weighted structural MR image with optimized gray/white matter contrast was acquired (3D MP-RAGE, 1 × 1 × 1 mm^3 voxels, TR = 2.730 s, TE = 2.95 ms, TI = 1000 ms, FOV = 256 mm, FA = 7°).

2.7. Behavioral task

The experimental paradigm that was performed during functional MRI scanning consisted of a blocked design, including a condition with angry and fearful face stimuli (http://www.macbrain.org) and a visuo-motor control condition with scrambled ellipse stimuli, a task specifically designed to elicit amygdala activity (Hariri et al., 2002; van Wingen et al., 2011). Two blocks of angry and fearful faces were interleaved with three control blocks, and each 30 s block consisted of six 5 s trials. Each trial consisted of three simultaneously presented stimuli, with the cue stimulus presented above the target and distractor. In the faces condition, an angry or fearful face was presented on top as cue, and participants had to indicate by an appropriate button press which of the bottom two faces (one angry and one fearful) matched the cue in emotional expression. In the control condition, a horizontally or vertically oriented ellipse was presented as cue above two ellipses (one vertical and one horizontal), and participants had to select the identically oriented ellipse.

2.8. Data analysis

Image analysis was performed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). The first five EPI-volumes were discarded and the remaining images were realigned to the first volume. Images were then coregistered to the anatomical scan, corrected for differences in slice acquisition time, spatially normalized to the MNI T1 template, resampled into 2 × 2 × 2 mm^3 voxels, and spatially smoothed (8 mm FWHM). Statistical analysis was performed within the framework of the general linear model (Friston et al., 1994). To assess the influence of combat stress on neural responsivity, the two experimental conditions were modeled as box-car regressors convolved with the canonical hemodynamic response function of SPM5. In addition, the realignment parameters were included to model potential movement artifacts, as well as a constant. Furthermore, a high-pass filter (cut-off 1/128 Hz) was included, and temporal autocorrelation was modeled with an AR(1) process. Contrast images subtracting the visuo-motor control condition from the emotion condition were obtained, and analyzed in subsequent random effects models.

Voxel-wise statistical tests were family-wise error (FWE) rate corrected ($p < 0.05$) for multiple comparisons across the entire brain, or for the search volume for regions of interest using a small volume correction (SVC) (Worsley et al., 1996). The search volume for the amygdala was anatomically defined using the WFU Pickatlas toolbox implemented in SPM5 (Maldjian et al., 2003). For the correlation and regression analyses with GR number, the imaging data were extracted from the amygdala mask (see Fig. 1). Pearson correlation analyses and linear hierarchical regression analysis were performed using PASW Statistics 18.0, $p < 0.05$ (two-tailed) was considered significant. Data were assessed for the presence of significant outliers. One of the participants had a GR number which deviated >2 SD from the mean and was therefore excluded from the analyses.

3. Results

3.1. Demographics

The participants that were included for the analysis were 22 male soldiers who had been deployed to Afghanistan.

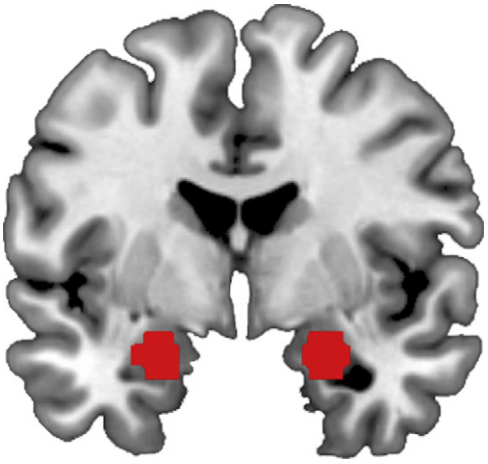

Figure 1 Amygdala mask used for extraction of imaging data.

Participant characteristics are reported in Table 1. We measured combat exposure during deployment to quantify stress exposure. The average score for combat exposure (mean ± SD; 5.4 ± 2.6) was comparable to that of a previously reported reference population of Gulf War veterans (4.0 ± 3.2) (King et al., 2006). None of the participants were physically injured during deployment.

Table 1 Participant characteristics.

Participants (n = 22)		
	Mean	SD
Age	24.0	6.6
BMI prior to deployment (kg/m^2)	24.2	1.4
Plasma cortisol prior to deployment (nmol/l)	410.23	207.12
ISCED level	2.5	0.5
Smoking (yes/no)	11/11	
Early life trauma (number of experiences)	3.0	2.1
Combat experiences	5.4	2.6
PTSD symptoms pre-deployment	27.6	4.7
PTSD symptoms post-deployment	27.9	6.2

ISCED — International Standard Classification of Education; early life trauma was measured with a checklist (Bremner et al., 2007). PTSD symptoms measured with the 22 item self-report PTSD scale (Hovens et al., 1994), range of possible scores 22—110. The change in PTSD symptoms was not significant (paired samples t-test, p = 0.860).

3.2. Behavioral performance

Prior to deployment, task accuracy was similar in both conditions (median ± IQR; faces condition: 100.0 ± 10, control condition: 94.4 ± 6; p = 0.279) but reaction times were slower in the faces condition (mean ± SD; faces condition: 2059 ± 474 ms, control condition: 1151 ± 260 ms; T = 9.76, $p < 0.001$). After deployment, task accuracy was also similar in both conditions (median ± IQR; faces condition: 100.0 ± 8, control condition: 100.0 ± 6; p = 0.529), and again reaction times were slower in the faces condition (mean ± SD; faces condition: 1994 ± 291 ms, control condition: 1140 ± 405 ms; T = 9.82, $p < 0.001$). There were no significant changes in accuracy (p = 0.199) or reaction time (p = 0.659) over time. In addition, the interaction of time - × condition was not significantly different for both accuracy (p = 0.528) and reaction time (p = 0.600).

3.3. Neural responsivity

To verify that the task indeed activated the amygdala, we compared the faces condition with the control condition across measurements. As expected, the task increased activity in the amygdala, as well as in the insula, dorsal anterior cingulate cortex (dACC), occipitotemporal cortex, hippocampus, orbitofrontal cortex, inferior and middle frontal gyri, thalamus, precuneus, angular gyrus, and cerebellum (all $p_{cor} < 0.05$; see Table 2). The increase in amygdala activity of individuals after deployment, was trend

Table 2 Brain regions with larger responses during the face matching condition than during the control condition (i.e., main effect of fMRI task).

	MNI coordinates			Cluster size	t
	x	y	z		
R/L inferior occipitotemporal cortex	42	−48	−20	8455	15.4
R inferior frontal gyrus	50	24	28	1227	11.6
R amygdala	20	−6	−14	222	11.5
L amygdala	−18	−6	−14	306	9.9
L inferior frontal gyrus	−46	18	32	706	9.1
L parahippocampal gyrus	−8	−30	−4	92	7.7
L middle temporal gyrus	−58	−46	8	112	7.3
R parahippocampal gyrus	16	−30	−2	98	7.2
Supplementary motor area	−2	14	54	49	6.9
L inferior parietal lobule	−32	−58	52	26	6.7
R calcarine gyrus	10	−74	6	52	6.7
L inferior frontal gyrus	−36	30	0	36	6.5
Precuneus	−4	−54	48	66	6.3

Data are local maxima for significant clusters ($p < 0.05$, FWE, cluster ≥ 20 voxels). Cluster size in number of voxels.

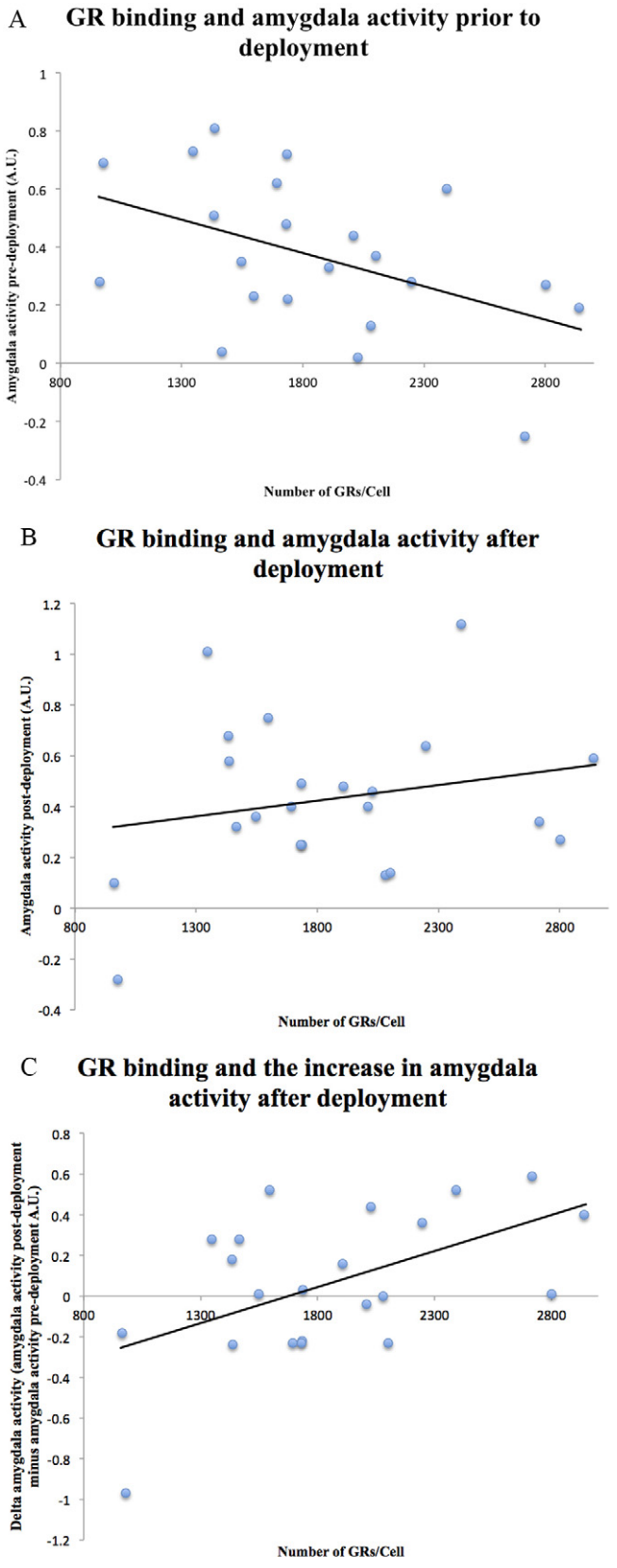

Figure 2 GR number in PBMCs prior to deployment is significantly correlated with amygdala activity prior to deployment (A) but not to amygdala activity after deployment (B). GR number is also significantly associated with the increase in amygdala activity after deployment (C).

significant, after small volume correction (peak Montreal Neurological Institute coordinate (*x*, *y*, *z*); (26, −2, −26), $Z = 2.9$, $p_{cor} = 0.076$).

3.4. GR number in PBMCs and amygdala activity

Pre-deployment GR number in PBMCs was significantly negatively correlated to pre-deployment amygdala activity (Pearson's $R = -0.465$, $p = 0.029$; see Fig. 2A), but not to post deployment amygdala activity (Pearson's $R = 0.213$, $p = 0.340$). The difference between the correlations of pre-deployment GR with pre- and post-deployment amygdala activation is significantly different (Steiger's $Z = 2.41$, $p = 0.016$). The partial correlation of GR and baseline amygdala activation, controlling for BMI was Pearson's $R = -0.468$, $p = 0.033$. More importantly, we also examined if GR number in PBMCs was associated with the amygdala response in reaction to severe stress. Pre-deployment GR number in PBMCs was significantly correlated to the increase in amygdala activity after deployment (Pearson's $R = 0.525$, $p = 0.012$; see Fig. 2B). In order to control for age, body-mass index (BMI), and combat experiences, we entered these into a linear hierarchical regression analysis with GR number. To illustrate the unique effects of the variables, these were entered in separate blocks (see Table 3). GR number was entered into the first block, age and BMI in the second block and combat experiences in the third block. Linear regression analysis significantly predicted the increase in amygdala activity after deployment (linear regression, $F = 3.931$, $p = 0.019$, $R^2 = 0.480$, adjusted R^2 0.358; see Table 3). Both GR number ($\beta = 0.571$, $p = 0.005$) and BMI ($\beta = 0.438$, $p = 0.034$) were significant predictors of the increase in amygdala activity after deployment. As expected, PTSD symptom severity change (within these healthy individuals with little variation in PTSD scores) was not correlated with amygdala activation change (Pearson's $R = 0.068$, $p = 0.794$).

To assess the anatomical specificity of the associations between GR number and amygdala activity, additional voxel-wise correlations were performed. Pre-deployment GR number was negatively correlated to amygdala activity (MNI coordinates (*x*, *y*, *z*); left: (−18, −6, −20), $Z = 3.3$, $p_{cor} = 0.026$; right: (22, −2, −22), $Z = 3.0$, $p_{cor} = 0.57$). Furthermore, pre-deployment GR number was positively correlated to the increase in amygdala activity after deployment (left: (−20, −2, −16), $Z = 2.9$, $p_{cor} = 0.077$; right: (20, −2, −20), $Z = 3.6$, $p_{cor} = 0.009$). No significant correlations with other brain regions were observed after correcting for multiple comparisons, suggesting that GR number primarily predicts amygdala functioning.

4. Discussion

In this study, we investigated whether peripheral GR number, which has been shown to be a predictor for the development of PTSD symptoms after combat exposure (van Zuiden et al., 2011, 2012), was related to amygdala functioning in healthy individuals exposed to severe stress. We assessed GR number in PBMCs in soldiers before deployment to Afghanistan. Amygdala functioning was assessed with functional magnetic resonance imaging (fMRI) in the same group of soldiers before and after deployment. The increase in amygdala activity

Table 3 Linear regression of age, BMI, and predeployment GR number in PBMCs on the increase in amygdala activity after military deployment ($N = 22$).

	Block 1 $F_{1,21} = 7.613^{*}$ $R^2 = 0.276$			Block 2 $F_{3,21} = 5.071^{**}$ $R^2 = 0.458$			Block 3 $F_{4,21} = 3.931^{***}$ $R^2 = 0.480$		
Variable	β	t	p	β	t	p	β	t	p
GR number	0.525	2.759	0.012	0.575	3.268	0.004	0.571	3.225	0.005
Age				−0.032	−0.171	0.866	−0.108	−0.516	0.613
BMI				0.441	2.340	0.031	0.438	2.309	0.034
Combat experiences							−0.168	−0.857	0.403

BMI, body mass index; GR, glucocorticoid receptor; PBMCs, peripheral blood mononuclear cells; all variables were forced into entry in three separate blocks to illustrate the unique effects of the variables. GR number was entered into the first block, age and BMI in the second block, and combat experiences in the third block.

* $p = 0.012$.

** $p = 0.010$.

*** $p = 0.019$.

after deployment was trend significant ($p = 0.076$). This was due to a lower number of participants in this study compared to our previous study (van Wingen et al., 2011). Pre-deployment GR number was significantly correlated with pre-deployment amygdala activity. More importantly, pre-deployment GR number significantly predicted a substantial proportion of variance in the increase in amygdala activity following deployment. Thus it appears that high GR number in PBMCs not only is a vulnerability factor for PTSD (van Zuiden et al., 2011, 2012), but may also lead to increased amygdala activity after experiencing severe stress. Interestingly, a recent study in mice with high trait anxiety revealed increased levels of hippocampal GR protein and mRNA expression under non-stressed conditions, compared to mice with low trait anxiety (Jakovcevski et al., 2011). In addition, systemic injection of the glucocorticoid receptor antagonist mifepristone (RU486) decreased the stress-induced activation of the HPA axis and the long-term anxiogenic effects of stress observed in mice with high trait anxiety. This suggests that a central GR pathway dysregulation may indeed be associated with increased vulnerability for the development of stress-related disorders.

It is well known that the amygdala plays an important role in threat detection and is part of a larger salience network (Davis and Whalen, 2001; Phelps and LeDoux, 2005; LeDoux, 2007). In a number of stress and anxiety related disorders, such as PTSD and panic disorder, amygdala hyperactivity is frequently found (Rauch et al., 2000; Shin et al., 2005; Etkin and Wager, 2007). It is thus of particular interest that GR receptor number (in PBMCs prior to deployment), which has previously been shown to be a predictor of PTSD symptoms (van Zuiden et al., 2011, 2012), also predicts the increase in amygdala activation after deployment. Earlier work by Kukolja et al. (2008) has shown that elevation of norepinephrine and cortisol to moderate stress levels induces an amygdala response bias to fear equivalent to amygdala hyperactivity observed in patients with PTSD. Administration of hydrocortisone sodium succinate (HCORT) led to increased activation of the amygdala in veterans with and without PTSD (Yehuda et al., 2009). However, in healthy individuals, the administration of hydrocortisone actually reduces amygdala responses (Henckens et al., 2010). It may be proposed that cortisol increases amygdala activity only in the presence of severe stress exposure, possibly through interaction with the noradrenergic system (Valentino et al., 1983, 1993). Paradoxically, although pre-deployment GR number was negatively correlated with pre-deployment amygdala activity, pre-deployment GR number actually predicted the increase in amygdala activity after deployment. The significance of this relationship is still unknown, although it could be speculated that GR regulation of amygdala activity may be altered due to exposure to severe stress. Assessment of post-deployment GR number could have shed more light on this issue. Unfortunately we have no data available on post-deployment GR number in these individuals.

Although it is unclear how pre-deployment GR number in PBMCs is involved in amygdala functioning, it may be hypothesized that GR number in PBMCs is a reflection of central GR function. Glucocorticoid receptors are found throughout the brain, particularly in limbic areas, such as the amygdala (Meaney et al., 1985; Groeneweg et al., 2011). Studies in rodents have shown that GR within the CNS plays an important role in fear conditioning (Kohda et al., 2007; Kolber et al., 2008) and extinction (Cai et al., 2006; Yang et al., 2006). Glucocorticoids thus appear to modulate the formation of emotional memories (see Rodrigues et al., 2009 for a review). Inactivation of GR within the amygdala leads to persistent disruption of traumatic memories (possibly indicating a novel direction for prevention of PTSD) (Tronel and Alberini, 2007).

Mechanisms of central and peripheral GR regulation are still poorly understood. In this study we made use of a peripheral measure of GR number, which may not be a genuine reflection of GR in CNS. However, research has shown that neuronal and lymphoid cytosolic GRs are similar in glucocorticoid affinity and specificity (Lowy, 1989). In addition, cytosolic GRs in both brain and peripheral immune tissue are down-regulated after administration of corticosterone following adrenalectomy (Lowy, 1991; Spencer et al., 1991). It is thus noteworthy that the current study demonstrates an association between peripheral GR and central brain function. The availability of a suitable radiotracer for GR would enable future studies to examine central GR function using SPECT or PET (Steiniger et al., 2008; Wuest et al., 2009).

The current study illustrates the power of combining diverse neurobiological techniques, to gain a broader understanding of mechanisms involved in exposure to severe stress and development of stress-related disorders. However, there are several limitations, which should be addressed. Caution should be used in interpreting functionality from correlational analyses, such as used in this study. The results of this study point to a potentially interesting mechanism in which glucocorticoid functioning could influence amygdala functioning possibly through altered fear conditioning and thus increase vulnerability for psychiatric disorders after exposure to severe stress. Future research should also examine the functional implications of increased GR number by taking into account functional assessment of the HPA axis (e.g. a dexamethasone suppression test). Unfortunately, this study examined these effects in a limited number of participants, thus these results should be replicated in a larger study. Although, none of the participants were physically injured during deployment, we did not assess actual blast exposure, thus we cannot exclude the possibility that this could have affected the results. The current study's strength, a homogenous sample, is also its weakness, as it limits the generalizability of the results. We only examined (relatively young) males, who were exposed to a similar prolonged severely stressful period. Future studies should examine the relation of GR and amygdala activity in females, individuals exposed to other stressful and traumatic events, and for differing lengths of time. In addition, studies should examine how GR and amygdala activity are related to the development of PTSD by assessing GR and amygdala activity in a larger prospective study of individuals exposed to trauma as well as in patients with PTSD.

In conclusion, our results demonstrate that GR number in PBMCs is related to amygdala activation in humans, and that GR number in PBMCs prior to exposure to severe stress predicts the increase in amygdala activity after exposure. Although it remains unclear how this relationship is mediated, this study has shown that it is a research topic that deserves further exploration.

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Conflict of interest

The authors have no potential conflicts of interest.

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