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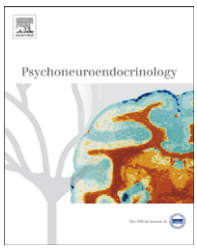

Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor

Simone Kern[a], Terrence R. Oakes[b], Charles K. Stone[d], Emelia M. McAuliff[b], Clemens Kirschbaum[c], Richard J. Davidson[b,*]

[a]*Klinik und Poliklinik für Neurologie, Technische Universität Dresden, Germany*
[b]*The Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, 1500 Highland Avenue, Madison, WI 53705, USA*
[c]*Institut für Biologische Psychologie, Technische Universität Dresden, Germany*
[d]*Department of Cardiology, University of Wisconsin, Madison, USA*

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Summary
The prefrontal cortex (PFC) has been well known for its role in higher order cognition, affect regulation and social reasoning. Although the precise underpinnings have not been sufficiently described, increasing evidence also supports a prefrontal involvement in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis.
Here we investigate the PFC's role in HPA axis regulation during a psychosocial stress exposure in 14 healthy humans. Regional brain metabolism was assessed using positron emission tomography (PET) and injection of fluoro-18-deoxyglucose (FDG). Depending on the exact location within the PFC, increased glucose metabolic rate was associated with lower or higher salivary cortisol concentration in response to a psychosocial stress condition. Metabolic glucose rate in the rostral medial PFC (mPFC) (Brodman area (BA) 9 and BA 10) was negatively associated with stress-induced salivary cortisol increases. Furthermore, metabolic glucose rate in these regions was inversely coupled with changes in glucose metabolic rate in other areas, known to be involved in HPA axis regulation such as the amygdala/hippocampal region. In contrast, metabolic glucose rate in areas more lateral to the mPFC was positively associated with saliva cortisol. Subjective ratings on task stressfulness, task controllability and self-reported dispositional mood states also showed positive and negative associations with the glucose metabolic rate in prefrontal regions.

Abbreviations: ACC, anterior cingulate cortex; IFG, inferior frontal gyrus; *maxinc_d*, difference in maximum cortisol increase for the stress and control condition; MFG, medial frontal gyrus; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; SFG, superior frontal gyrus.

*Corresponding author.
E-mail address: rjdavids@wisc.edu (R.J. Davidson).

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These findings suggest that in humans, the PFC is activated in response to psychosocial stress and distinct prefrontal metabolic glucose patterns are linked to endocrine stress measures as well as subjective ratings on task stressfulness, controllability as well as dispositional mood states.

1. Introduction

The hypothalamus–pituitary–adrenal (HPA) axis is a hierarchically organized stress system, involved in the organism's adaptation to aversive conditions. Activation of the HPA axis results in secretion of glucocorticoids, which are known to have far reaching adaptive effects on the organism's metabolism, immune and central nervous system (Sapolsky et al., 2000). Central stress circuits orchestrate the activation of the HPA axis (Herman and Cullinan, 1997), though the precise details about the circuitries and brain regions involved in this regulatory process are not completely known.

In rats (Diorio et al., 1993), and especially in primates (Sanchez et al., 2000), there is a high density of glucocorticoid receptors in medial prefrontal cortex (mPFC). In these same regions of the mPFC, stress-induced increases in immediate early gene expression (Figueiredo et al., 2003), and dopamine concentration (Sullivan and Gratton, 1998), support the notion that the mPFC, with its distinct functions in higher order processing and its various ascending and descending projections (Carmichael and Price, 1995), plays a crucial role in HPA axis regulation. Lesions in the mPFC of rats significantly increase adrenocorticotropic hormone (ACTH) and corticosterone secretion due to restraint stress (Diorio et al., 1993; Figueiredo et al., 2003). Implants of crystalline corticosterone in the same region result in significantly decreased levels of ACTH and corticosterone due to restraint stress (Diorio et al., 1993). However, whereas dorsal regions of the mPFC seem to have an inhibitory influence on HPA axis function, there is evidence that ventral parts of the mPFC might have an excitatory impact on the axis (Sullivan and Gratton, 1999). More support for the regulatory role of the mPFC during stress exposure emerges from recent rodent data indicating that the mPFC is involved in mediating effects of uncontrollable and controllable stress, whereby the ventral mPFC seems to inhibit serotonergic activation in the dorsal raphé nucleus in the face of controllable stressors (Amat et al., 2005). However, all these reports are exclusively based on rodent models and little is known about the mPFC's role during stress exposure and its possible inhibitory or excitatory impact on HPA axis regulation in the primate brain. There are only a few studies directly investigating neural circuits of stress in humans (Critchley et al., 2000; Pruessner et al., 2004; Soufer et al., 1998), but so far, only one has specifically focused on neural substrates of HPA axis activation (Wang et al., 2005).

In summary, previous findings in animals (Diorio et al., 1993; Figueiredo et al., 2003) and humans (Wang et al., 2005) indicate positive as well as negative associations between prefrontal regions and the endocrine stress response but so far, no conclusive pattern of a distinct PFC involvement in neuroendocrine stress control has been established in humans. We hypothesize that depending on the exact location within the PFC, positive as well as negative associations between stress-induced glucose metabolic rate and saliva cortisol concentrations are present. While negative associations are expected to be located in the medial dorsal PFC (Diorio et al., 1993; Sullivan and Gratton, 2002), positive associations are expected in more lateral PFC regions (Wang et al., 2005). Following the idea of anatomically and functionally coupled stress circuitries involving prefrontal as well as limbic regions (Carmichael and Price, 1995; Herman and Cullinan, 1997), it is also hypothesized that stress-induced glucose metabolic changes in prefrontal regions relate to metabolic patterns in limbic structures.

In order to test these hypotheses, the experiment presented herein was designed to specifically activate the HPA axis in order to identify the neural circuitry involved in the regulation of the axis with a specific focus on the prefrontal cortex. Stressors that include components of social threat and/or uncontrollability are most potent when it comes to HPA axis activation (Dickerson and Kemeny, 2004). A well-validated psychosocial stress test incorporating these components was therefore chosen. A control condition was devised that closely matched the stress condition but which lacked the social stress element and thus did not activate the HPA axis. In order to evaluate the effectiveness of the stress vs. the control condition, salivary cortisol samples were collected throughout the entire experiment. Following the idea that the PFC plays an integrative role in cognitive and affective processing (e.g. emotion regulation) (Ochsner et al., 2002; Urry et al., 2006), psychometric measures assessing subjective ratings on the perceived task stressfulness, perceived controllability, and dispositional mood states, were administered to gain further insight into how neural substrates of stress relate to psychological domains.

2. Methods and material

2.1. Participants

Fourteen male human subjects, recruited by posting flyers at university buildings, participated in the study. Participants were between 18 and 23 years old with a mean age of 20.5 years (S.D. $\pm$1.91 years).

All participants were screened on the phone and reported to be right handed (Chapman and Chapman, 1987) and non-smokers. People who reported a history of psychoactive substance use, head trauma, neurological, psychiatric, allergic, metabolic or cardiovascular disorder were excluded. People with previous experience of claustrophobia

or fear of needles or blood were excluded, too. If eligible, participants were invited for their first session.

2.2. Experimental procedure

Each participant reported to the laboratory for a total of three sessions. Session 1 was a simulation session. Written informed consent was obtained at the beginning of this session and the study procedure was explained in full detail. Approximately 14 days after the first visit, each participant underwent two positron emission tomography (PET) scans separated by exactly 1 week. PET scans for each participant were conducted at the exact same time of day. All scans were performed in the afternoon between 12:00 and 16:30 h. Participants were requested to fast for 4–5 h prior to the experiment. Following a cross over design, half of the subjects were randomly assigned to have the stress procedure at their first PET scan and the control procedure at their second PET scan. The other seven participants had the reversed order. Self-report ratings on perceived stressfulness, controllability and related domains were obtained immediately after the stress as well as the control condition (visual analog scales).

The study was reviewed and approved by the University of Wisconsin–Madison Human Subject Committee.

2.3. Stress condition

The stress condition used in this experiment was a modified version of the "*Trier Social Stress Test*" (*TSST*) (Kirschbaum et al., 1993). The TSST is a psychosocial stress test consisting of 3 min of preparation time, 5 min of free speech and 5 min of mental arithmetic in front of two panel members and a camera (described in detail elsewhere). The TSST was chosen as it incorporates elements of psychosocial threat and uncontrollability and it has been shown to promote very robust activation of the HPA axis and to induce stronger increases in salivary cortisol than any other laboratory stress test known at this point (Dickerson and Kemeny, 2004).

In order to occupy the bulk of the initial fluoro-18-deoxyglucose (FDG) up-take, another 5-min speech task (word definition) as well as another 5 min of mental arithmetic were added to the original TSST design. For the speech task participants were requested to describe their qualification on a given job position. The word definition task requested verbal definitions for words read out loud by the panel members (e.g. Tell us the meaning of "opaque"). The first math task required counting backwards loudly from 2043 in steps of 17. For the final math task the subject had to start with the number 5, add 3 and then multiply the result with 2. Each task lasted exactly 5 min and was performed in front of the two panel members. After the stress procedure was over, participants were guided back to the PET preparation room.

2.4. Control condition

The control condition was designed to match the stress condition without inducing stress. In order to reduce the stressfulness of the situation, we removed the camera as well as the presence of the panel from the original design. In an initial pilot study, we could show that removal of the panel as well as the camera from the TSST setting prevented an activation of the HPA axis (data not published).

The control condition involved the following tasks: participants were asked to give a spontaneous speech (about a movie, a trip or a book), followed by a word definition task (e.g. "Tell us the meaning of 'happy'".). Words were recorded on tape and played to the participant. Next, each participant was asked to count backwards from 5000 in steps of 7. Finally each subject was requested to start with the number 1, add 1 and then multiply the result by 2. Each task lasted for exactly 5 min. Participants were alone in the room for this condition. The investigator entered the room only to give instructions between the tasks. A hidden camera in the shape of a radio clock was placed in the room in order to verify that each participant followed the instructions while being alone in the room.

At the end of the study each participant was debriefed about the existence of the hidden camera and written consent for usage of the tapes was obtained. Tapes were not inspected prior to when written consent was given. Consent was given by all 14 subjects. Post-experiment inspections of the tapes showed that all subjects followed the given instructions.

2.5. Questionnaires

Participants were asked to fill out several questionnaires assessing subjective ratings on affectivity and situational stress ratings.

The Mood and Anxiety Symptom Questionnaire (MASQ) (Watson et al., 1995) was given at the first of the three visits. During visits 2 and 3, an in-house questionnaire (visual analog scales 0–100) assessing subjective ratings on situational stressfulness (e.g. "I experienced the interview to be stressful."), controllability (e.g. "During the interview, I had total control over the situation.") and perceived threat (e.g. "The situation was very threatening.") was completed immediately following both the stress and control condition respectively.

2.6. PET and MRI scans

After arrival at the laboratory, participants were seated in a quiet, dim preparation room and an intravenous catheter was inserted into the cubital veins of the left and the right arm. After successful insertion of the catheters, participants were allowed to rest for 60 min. Questionnaires were given during this period. After the resting period, a baseline saliva sample was collected, followed by an injection of 5 mCi of FDG. Each saliva sample was accompanied by a blood sample. Immediately after FDG injection, the subject was guided to a nearby room where either the stress test or the control condition was performed. After completion of the experimental condition, participants were guided back to the PET preparation room and the first of four post-treatment saliva samples was collected (30 min post-injection). Each subject then filled out an in-house questionnaire assessing the stressfulness of the situation. Two more samples were collected 40 and 50 min post-injection. After the third post-treatment sample was collected (50 min after

FDG injection), subjects were positioned on the scanner bed and the scan was initiated.

After the scan, a final saliva sample was collected (110 min post-injection) and the catheters were removed.

The second PET scan took place exactly 1 week after the first scan. The procedure for PET scan 2 mimicked the procedure for scan 1. The only difference that occurred was the nature of the experimental condition: if the stress condition was administered at the first scan, participants had the control condition at their second scan (or visa versa). At the end of session 3, after removal of the catheters and after a light snack was served, participants were guided to the local MRI scanner and a high-resolution anatomical scan was performed (details below).

2.7. PET scan acquisition

Fluoro-18-deoxyglucose (Eastern Isotopes, Milwaukee) was used as a tracer of local cerebral metabolism. A dose of 5 mCi per scan was injected. PET data were acquired using a General Electric/Advance PET scanner (DeGrado et al., 1994). This scanner has an intrinsic resolution of 5–6 mm full-width at half-maximum (FWHM), and a reconstructed resolution of 8–10 mm FWHM for a brain positioned near the center of the field of view. The scan started approximately 50 min after injection, and consisted of a set of three, 10-min emission scans followed by a 15 min transmission scan. Images were reconstructed to $1.75 \times 1.75 \times 4.25\,mm^3$ voxels using the manufacturer's software and incorporating corrections for deadtime, random events, detector normalization, scatter and attenuation. The transmission scan was used as the input for an automatic segmented attenuation correction using the scanner software.

2.8. MRI scan acquisition

MRI structural images were acquired for anatomical localization of functional activity. For this purpose an axial 3D SPGR (TE = 1.8 ms, TR = 8.9 ms, flip angle = 10°, FOV (field of view) = 256 mm × 256 mm, 124 slices, slice thickness = 1.2 mm) was acquired on a 3 T GE Sigma MRI.

2.9. PET data processing and analysis

PET data were analyzed using the SPM2 (Statistical Parametric Mapping) software package (Wellcome Department of Cognitive Neurology, London) and in-house software Spamalize. After initial visual inspection, PET emission data were corrected for inter-frame motion, summed across frames, and coregistered into a standard stereotaxic space (MNI) (Evans et al., 1993). For the latter step, the high-resolution three-dimensional T1 MRI scans were first coregistered to the MNI template using a 12-parameter affine fit. Then the summed PET images were coregistered to the corresponding MRI T1 scan for each subject using a six-parameter (rigid-body) fit, and finally the transform matrices were cumulated and the PET images were brought into register with MNI template. After coregistration, PET data were smoothed with a Gausian kernel (FWHM = 8 mm). In order to control for between-injection and between-subject variability in mean glucose utilization, each voxel was globally normalized by the mean voxel value using the SPM2 global normalization tool to achieve a global mean of 5. The resulting images were then scaled to the group mean value by applying the SPM2 tool for grand mean scaling. Glucose metabolism was not further quantified as repeated arterial blood sampling right after tracer injection would have interfered with the study design.

PET data from the stress and control condition were statistically compared using a paired *t*-test (population main effect) via SPM2 (Friston et al., 1991). Globally normalized, grand-mean-scaled and thresholded difference images (stress–control) were created for each subject. This difference image along with the regression variables of interest (e.g. *maximum cortisol rise*, *perceived stressfulness*, *perceived controllability)* were entered in a single subject–single covariate voxel-wise whole brain correlation (SPM2).

Initial regression analysis for *maximum cortisol rise*, perceived stressfulness, perceived controllability and dispositional mood states (e.g. MASQ: general distress, depressive symptoms) were restricted to prefrontal areas. Prefrontal areas were differentiated from supplementary motor areas, by defining the coronal plane that bi-sected the distance between the cingulate sulcus and pre-central sulcus (Convit et al., 2001). The coronal plane (−8.4 mm) was defined according to a Talairach Standardized Brain Atlas (Mai et al., 2004). The corresponding coordinate was then transformed into MNI space ($y = 9$). Coronal planes equal or anterior to this coordinate where defined as prefrontal region.

After the regression analysis, ROI masks for the significant PFC clusters were created and values for the maximum grand mean scaled FDG concentrations (referred to as *glucose metabolic rate*) within these clusters were extracted for each subject in order to examine the data for outliers. Spamlize's BrainMaker ROI (region of interest) tool was used for this step. In order to test for correlational associations of these PFC clusters with other brain regions, extracted cluster values were next entered in a single subject–single covariate voxel-wise whole brain correlation analysis (SPM2).

Regression analysis restricted to prefrontal areas are shown at $p \leqslant 0.005$ uncorrected for multiple comparisons. Whole brain analysis (*t*-test and regression) is shown at $p \leqslant 0.001$ uncorrected for multiple comparison. All clusters were reported for $k > 10$ voxels.

In order to test for hemispheric asymmetry of significant PFC clusters, values for glucose metabolic rate from homologous clusters in the opposite hemisphere were extracted (Spamalize; BrainMaker ROI). In a subsequent step, values were then entered in a correlation analysis (SPSS 11).

2.10. Salivary cortisol sampling and analysis

A total of five saliva samples were collected. Collection times were 1 min prior to FDG injection as well as 30, 40, 50 and 110 min post-injection. Salivettes® (Saarstedt, Germany) were used for specimen collection. Salivettes® were stored at −20 °C until samples were analyzed for salivary cortisol (nmol/l) using a luminescent immuno assay

(IBL, Hamburg/Germany). The intra-assay and inter-assay variability have been shown to be $<8\%$, respectively.

A score of maximum salivary cortisol increase (*maxinc*) was calculated for each subject and for each condition by subtracting the baseline sample from the maximum post-treatment sample (sample 3 minus sample 1). The difference between *maxinc* for the stress condition and *maxinc* for the control condition was then calculated (*maxinc_d*) and used as a covariate in a voxel-wise whole brain correlation. All statistical analyses were performed using SPSS 11 for Macintosh OS X.

3. Results

3.1. Endocrine and behavioral data

Salivary cortisol concentrations were significantly different between the stress and control condition (main effect for stress/control condition: $F(1,13) = 6.36$, $p = 0.026$; interaction of condition $\times$ sample: $F(4,52) = 6.35$ $p = 0.011$; see Figure 1).

Maximum salivary cortisol increase (*maxinc*) during the stress condition was significantly higher than during the control condition (paired *t*-test: mean maxinc stress $=$ 15.25 ± 16.33 nmol/l; mean maxinc control $= 2.53 \pm 5.59$ nmol/l) ($t(13) = -2.80$; $p = 0.015$). The order of the condition (control condition first vs. stress condition first) had no significant effect on *maxinc* during either condition (two-factor (*order* $\times$ *condition*) repeated measure ANOVA analysis: interaction of *order* $\times$ *condition*: $F(1,12) = 0.18$; $p = 0.68$).

Participants perceived significantly more stress during the stress vs. control condition (paired *t*-test: $t(13) = 3.34$; $p = 0.005$). The stress condition was also perceived significantly less controllable (paired *t*-test: $t(13) = -4.21$; $p = 0.001$).

Difference in maximum increase between the stress and the control condition (*maxinc_d*) was associated with perceived stressfulness, perceived control and dispositional mood states (MASQ) (Table 1).

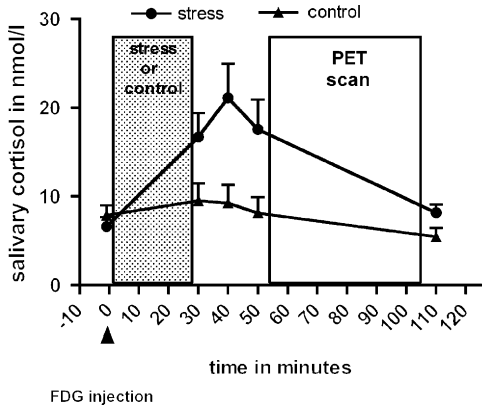

Figure 1 Salivary cortisol in nmol/l shown for the stress and control condition. Timing for FDG injection, experimental condition and scans is indicated. Error bars reflect standard errors of the mean.

3.2. PET data

The stress condition was associated with pronounced changes in glucose metabolic rate in prefrontal regions whereas the control condition resulted in only minor metabolic changes in this brain area. Moreover, for the stress condition (stress–control) but not for the control condition (control–stress), significantly elevated glucose metabolic rates in the mPFC (stress–control) were observed (see Table 1 in Supplementary Section).

3.3. PET data and endocrine stress measures

Next, associations between the regional difference in glucose metabolic rate between the stress and control condition (stress–control difference image) and the difference in maximum salivary cortisol increase (stress–control) (*maxinc_d*) were examined on a voxel-wise basis. In the mPFC, strong inverse correlations were found for *maxinc_d* and the difference in glucose metabolic rate between the stress and control conditions. These associations were most pronounced in two specific regions of the right rostral medial superior frontal gyrus (SFG) (Brodman area (BA) 9 ($r = -0.75$; $p = 0.002$) and BA 10 ($r = -0.80$; $p = 0.001$); see Figure 2). Negative associations were also observed for more dorsal aspects of the SFG, the medial frontal gyrus (MFG) and the inferior frontal gyrus (IFG) (Table 2). The direction of these correlations indicates that those individuals with greater increases in glucose metabolic rate in the mPFC in response to the stress vs. control conditions showed the lowest levels of salivary cortisol increase during the stress vs. control condition.

While the changes in glucose metabolic rate in the medial SFG was inversely correlated with *maxinc_d*, glucose metabolic rate in more lateral aspects of the SFG were positively associated with increases in salivary cortisol (*maxinc_d*) (right SFG: $r = 0.81$; $p < 0.001$). Positive associations were also observed for the anterior cingulate cortex (ACC) (ACC (BA 32): $r = 0.68$; $p = 0.007$) and the MFG ($r = 0.71$; $p = 0.004$) (Table 2).

Next we wanted to test whether the observed association between glucose metabolic rate in BA 9 and BA 10 was strictly lateralized. Therefore, we extracted values for glucose metabolic rate from the homologous clusters in the opposite hemisphere. Values were then entered in a correlation analysis. Glucose metabolic rate in the homologous clusters was not significantly associated with *maxinc_d*, indicating that negative associations between *maxinc_d* and glucose metabolic rate are restricted to BA 9 and B 10 in the right hemisphere. This test for hemisphere asymmetry was also performed for clusters positively associated with *maxinc_d* (e.g. SFG, MFG and ACC) but no significant association between glucose metabolic rate in the homologous cluster and *maxinc_d* was observed (correlation coefficients are listed in Table 2 in Supplementary Section).

3.4. Prefrontal associations

We next wished to identify other components of the circuit with which the mPFC regions were functionally coupled.

Table 1 Associations between *maxinc_d* (maximum cortisol increase stress–control), perceived stressfulness (stress–control), perceived control (stress–control) and dispositional mood states (MASQ).

	Perceived stressfulness (stress–control)	Perceived control (stress–control)	MASQ general distress	MASQ depressive symptoms	MASQ anhedonic symptoms	MASQ anxious arousal	MASQ anxious symptoms
maxinc_d stress–control	$r = 0.60$ $p = 0.024$	$r = -0.59$ $p = 0.026$	$r = 0.72$ $p = 0.003$	$r = 0.53$ $p =$ n.s.	$r = 0.33$ $p =$ n.s.	$r = -0.21$ $p =$ n.s.	$r = 0.22$ $p =$ n.s.

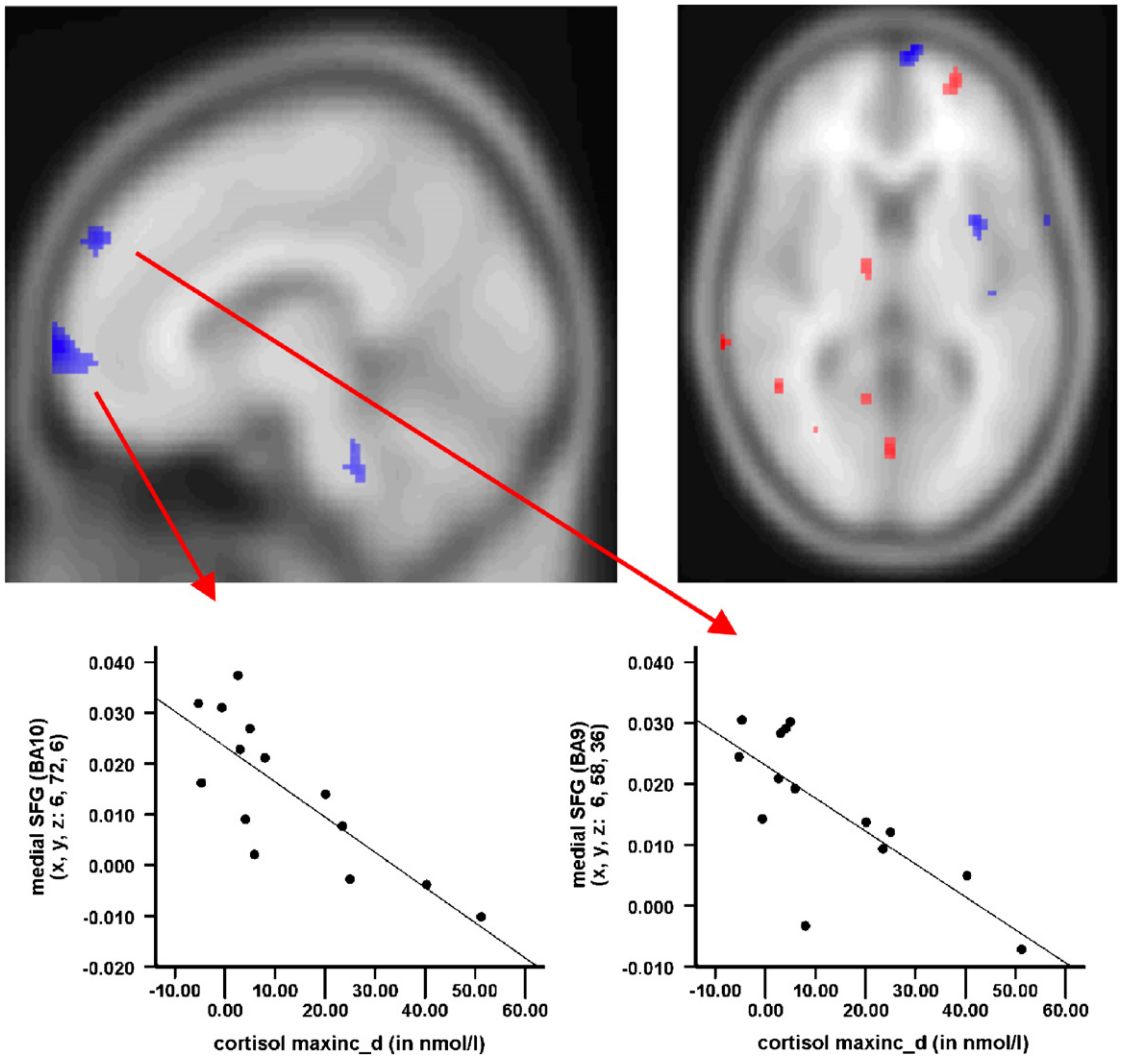

Figure 2 Voxel-wise whole brain correlation for *maxinc_d* and the corresponding difference image (*stress–control*). PET data are shown for $p<0.005$ uncorrected. Clusters are reported for $k>10$. Analysis was restricted to prefrontal areas ($y\geqslant 9$). Regions of interest were drawn on the basis of the activated cluster and glucose metabolic rates for BA 9 and BA 10 were extracted for each participant and plotted against each participant's *maxinc_d* score (BA 9: *x*, *y*, *z*: 6, 58, 36; $r = -0.75$; $p = 0.002$; BA 10: *x*, *y*, *z*: 6, 72, 6: $r = -0.80$; $p = 0.001$).

On the basis of the difference image between the stress and control conditions, separate voxel-wise whole brain correlational analyses were performed with the maximum glucose metabolic rate within each of the two stress-control mPFC clusters (BA 9 and BA 10 clusters). Area 9 showed a negative correlation with the left amygdala/hippocampal region (*x*, *y*, *z*: −28, −8, −32; $T = 7.82$; 464 mm^3; $r = -0.89$; $p<0.001$; Figure 3), the left pallidum (*x*, *y*, *z*: 20, −4, 4; $T = 7.72$; 352 mm^3; $r = -0.78$; $p = 0.001$), the left precuneus (*x*, *y*, *z*: −6, −50, 48; $T = 5.73$; 132 mm^3; $r = -0.84$; $p = 0.001$), and the left inferior OFG (*x*, *y*, *z*: −50, 42, −8; $T = 5.42$; 112 mm^3; $r = -0.77$; $p = 0.003$). Area 10 was negatively associated with the glucose metabolic rate in the left precuneus (*x*, *y*, *z*: −4, −58, 42; $T = 7.79$; 368 mm^3;

Table 2 This table lists brain areas, MNI coordinates, maximum *T* scores and cluster volumes in mm^3 for clusters based on a voxel-wise correlation between *maxinc_d*, self-report measures and brain activation (stress–control).

Positive correlation				Negative correlation			
Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*	Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*
maxinc_d							
MFG*	−32, 10, 50	*384*	**5.97**	Medial SFG* (BA 10)	6, 72, 6	*976*	**6.01**
SFG*	24, 58, 4	*440*	**5.67**	Medial SFG* (BA 9)	6, 58, 36	*584*	**5.47**
ACC* (BA 32)	−10, 40, −8	*96*	**3.49**	MFG	30, 18, 32	*296*	**5.41**
				Dorsal SFG	−14, 14, 70	*928*	**4.67**
				MFG	34, 58, 24	*128*	**4.62**
				IFG	−48, 14, 28	*272*	**4.28**
				Medial SFG	0, 32, 64	*528*	**4.23**
				OFG	32, 62, −6	*224*	**3.95**
				MFG	44, 14, 56	*88*	**3.60**
Stressfulness							
OFG	16, 30, −16	*112*	**5.05**	ACC (BA 32)	0, 36, 14	*352*	**5.34**
SFG	20, 50, 2	*368*	**4.68**	Medial SFG (BA 9)	6, 54, 38 (20, 38, 50)	*1632*	**5.05**
IFG	−48, 46, −12	*176*	**4.49**	IFG	44, 30, 16	*112*	**5.17**
Anterior insula	−36, 30, 2	*248*	**4.46**	MFG	−32, 28, 22	*224*	**4.99**
				MFG	−44, 12, 60	*320*	**4.95**
OFG	−16, 54, −22	*88*	**3.50**	Dorsal SFG	−18, 18, 64	*672*	**4.70**
				IFG	−32, 52, −16	*616*	**4.58**
				IFG	−50, 14, 30	*480*	**4.12**
				Dorsal SFG	18, 24, 66	*88*	**4.04**
				MFG	42, 18, 52	*288*	**4.02**
				IFG	−34, 10, 26	*88*	**4.00**
				MFG	−42, 58, 10	*104*	**3.92**
				MFG	32, 18, 34	*104*	**3.57**
Controllability							
Lateral IFG	−32, 56, 10	*560*	**6.10**	ACC (BA 32)	−8, 38, −10	*624*	**7.02**
Dorsal SFG	8, 16, 60	*232*	**5.15**				
Dorsal ACC (BA 24)	−12, 20, 34	*288*	**4.96**				
ACC (BA 32)	6, 34, 2	*264*	**4.81**				
Dorsal SFG	22, 32, 50	*464*	**4.57**				
MFG	32, 20, 30	*104*	**4.20**				
Medial SFG (BA9)	6, 56, 38	*184*	**4.05**				
IFG	−52, 22, 26	*144*	**3.78**				
SFG	10, 28, 64	*88*	**3.73**				
ACC (BA 32)	4, 36, 16	*144*	**3.61**				
MFG	−22, 48, 16	*104*	**3.58**				

Analysis was restricted to prefrontal areas ($y \geqslant 9$). Clusters were checked for laterality by extracting the maximum metabolic rate for the homologues cluster in the opposite hemisphere.

*Lateralized clusters. Data are shown for $p \leqslant 0.005$ uncorrected. All clusters are reported for $k > 10$ voxels.

$r = -0.79$; $p = 0.001$), the left fusiform gyrus (*x*, *y*, *z*: −42, −56, −14; $T = 6.23$; 112 mm^3; $r = -0.87$; $p < 0.001$), and the left medial temporal gyrus (*x*, *y*, *z*: −62, −8, −16; $T = 4.64$; 104 mm^3; $r = -0.79$; $p = 0.001$).

In order to test if the variances explained are significantly different across the neural variables, we performed a test for significance of difference between the correlation coefficient but no significant difference was observed for associations of BA 9 and B 10 (Table 3 in Supplementary Section).

The direction of all of these effects indicates that individuals who exhibit larger increases in glucose metabolism in BA 9 and BA 10 during stress compared with control conditions, exhibit smaller increases in glucose metabolism during these conditions in the stated regions.

BA 9 and BA 10 also showed positive correlation with other brain regions. These data are reported in Supplementary Section. Whole brain associations for PFC clusters positively associated with *maxinc_d* are also listed in Supplementary Section (Table 4).

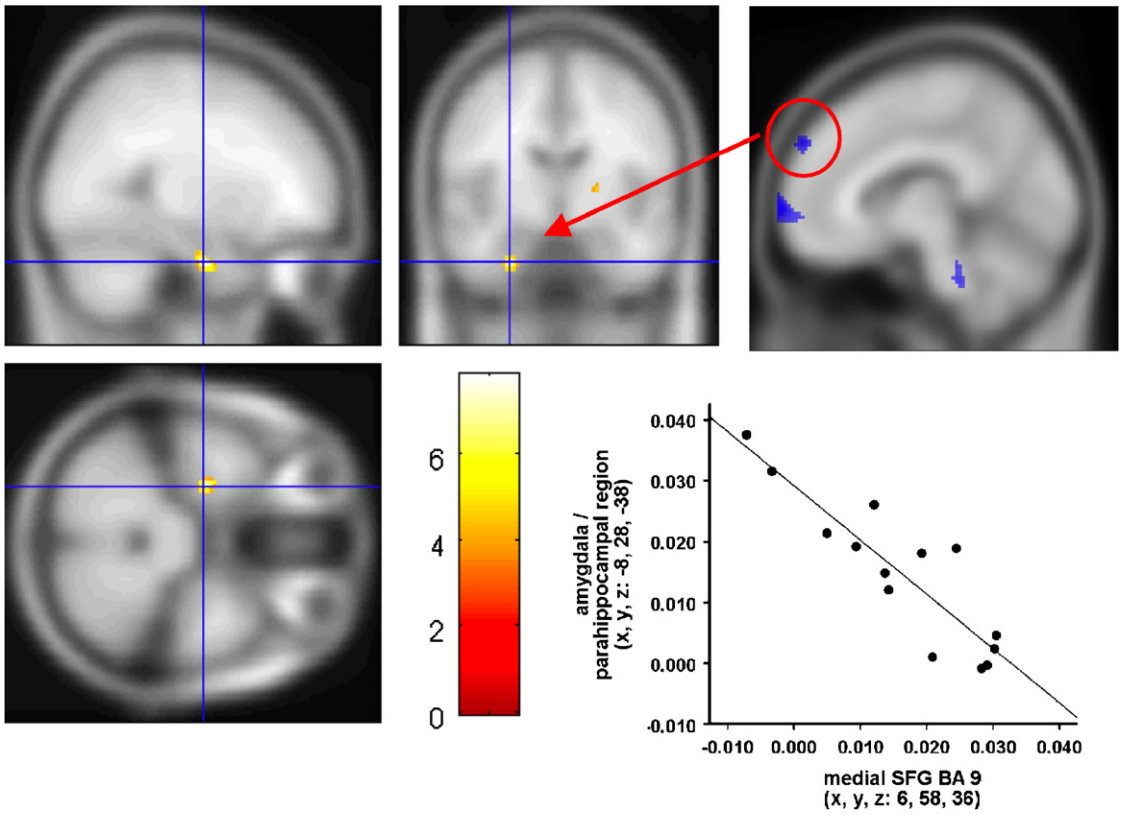

Figure 3 Glucose concentration for BA 9 and BA 10 were entered as covariates in a voxel-wise whole brain correlation. The glucose concentration in BA 9 showed an inverse relationship with the corresponding value in the left amygdala/hippocampal area. PET data are shown for $p<0.001$ uncorrected.

3.5. PET data, behavioral measures and dispositional mood states

Ratings on task stressfulness and controllability as well as self-reported dispositional mood states such as general distress and depressive symptoms were associated with distinct patterns of stress-induced prefrontal changes in glucose metabolic rate (Tables 2 and 3).

Partial overlap was observed when comparing metabolic patterns of endocrine and behavioral factors: changes in glucose metabolic rate in BA 9 were negatively associated with *maxinc_d* (*x*, *y*, *z*: 6, 58, 36), perceived stressfulness (*x*, *y*, *z*: 6, 54, 38 (20, 38, 50)), and reported general distress (*x*, *y*, *z*: 6, 60, 38). At the same time, glucose metabolic rate in this region was positively associated with perceived controllability (*x*, *y*, *z*: 6, 56, 38). This regional overlap is consistent with the analysis of the behavioral data showing significant correlations between these variables. However, the association between *maxinc_d* and changes in glucose metabolic rate in BA 9 remained significant even when perceived stressfulness (BA 9: *x*, *y*, *z*: 6, 60, 34; 192 mm^3; $T = 3.93$) or reported general distress (BA 9: *x*, *y*, *z*: 0, 54, 28; 520 mm^3; $T = 5.53$) were included in the model as an additional covariate. The same was true when perceived controllability was added as an additional covariate (BA 9: *x*, *y*, *z*: 6, 60, 36; 192 mm^3; $T = 3.96$).

This finding indicates that a change in metabolic glucose rate in BA 9 is primarily associated with stress-induced cortisol levels. Although behaviorally linked, perceived stressfulness and perceived controllability were associated with distinct prefrontal association patterns other than BA 9 and BA 10.

4. Discussion

Our findings indicate that in response to a psychosocial stressor, increased glucose metabolic rate in the mPFC areas BA 9 and BA 10 is inversely associated with stress-induced salivary cortisol concentrations. These findings suggest that the mPFC is engaged as part of regulatory circuitry to modulate the response to a stressful stimulus. While these data are consistent with the view that some regions of the PFC, particularly medial regions, modulate HPA axis functioning in an inhibitory fashion (Diorio et al., 1993; Sullivan and Gratton, 2002), a high glucose metabolic rate in more lateral aspects of the superior frontal gyrus are associated with increased cortisol levels, which is consistent with previous reports on PFC involvement in HPA axis regulation (Wang et al., 2005). This lateral PFC finding is also in accordance with data showing that unpleasant feelings which arise in connection with social interactions are associated with patterns of right prefrontal EEG activity (Davidson et al., 2000). This follows the idea of a right prefrontal asymmetry in negative affect and withdrawal behavior (Davidson and Irwin, 1999; Hewig et al., 2004). Accordingly, in rhesus monkeys (Kalin et al., 1998) and humans (Buss et al., 2003), right EEG asymmetry has been

Table 3 This table lists brain areas, MNI coordinates, maximum *T* scores and cluster volumes in mm^3 for clusters based on a voxel-wise correlation between self-report measures of dispositional mood states (MASQ) and brain activation (stress–control).

Positive correlation				Negative correlation			
Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*	Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*
General distress (MASQ)							
MFG	−30, 16, 54	*624*	**6.48**	IFG	−48, 12, 30	*600*	**6.81**
MFG	26, 30, 36	*168*	**4.68**	IFG	−56, 32, 12	*496*	**6.78**
OFG	12, 44, −26	*136*	**4.10**	SFG	−10, 22, 38	*112*	**4.56**
				MFG	32, 66, −6	*136*	**4.45**
				IFG	−40, 56, −8	*168*	**4.30**
				OFG	−8, 54, −16	*96*	**4.28**
				Gyrus rectus	0, 18, −18	*104*	**4.24**
				Dorsal SFG	−12, 16, 70	*680*	**4.15**
				Medial SFG (BA 10)	−6, 72, −4	*88*	**3.89**
				Dorsal SFG	−2, 26, 64	*88*	**3.83**
				SFG (BA 9)	6, 60, 38;	*104*	**3.83**
				MFG	−38, 14, 46	*88*	**3.70**
				Anterior insula	−46, 26, −8	*88*	**3.47**
Depressive symptoms (MASQ)							
OFG	10, 44, −26	*408*	**5.22**	Dorsal SFG	10, 42, 48	*728*	**7.36**
SFG	14, 38, −4	*352*	**5.04**	IFG	−60, 14, 28	*592*	**5.53**
MFG	−28, 14, 52	*280*	**4.35**	Dorsal SFG	16, 26, 68	*112*	**5.39**
ACC (BA 32)	−14, 44, −6	*152*	**4.23**	IFG	−56, 30, 8	*176*	**5.03**
Medial SFG (BA 9)	−4, 56, 16	*120*	**4.19**	ACC (BA 32)	0, 38, 10	*296*	**4.78**
OFG	18, 30, −16	*112*	**3.98**	Dorsal SFG	−16, 22, 66	*1096*	**4.69**
				OFG	−8, 68, −4	*216*	**4.59**
				MFG	−46, 34, 44	*232*	**4.58**
				Gyrus rectus	10, 18, −12	*152*	**4.54**
				Gyrus rectus	−2, 18, −20	*152*	**4.47**
				OFG	−46, 24, −8	*336*	**4.45**
				IFG	−42, 54, −16	*248*	**4.20**
				OFG	−34, 28, −22	*160*	**4.10**
				SFG	6, 12, 60	*512*	**4.03**
				Dorsal SFG	0, 26, 62	*104*	**3.95**
				Dorsal SFG	−24, 46, 48	*120*	**3.82**
				MFG	42, 20, 58	*88*	**3.82**
Anxious arousal (MASQ)							
MFG	48, 24, 36	*168*	**5.82**	OFG	32, 28, −22	*144*	**5.54**
MFG	−28, 32, 52	*288*	**4.86**	Insula	34, 16, 0	*632*	**4.80**
MFG	34, 30, 22	*232*	**4.37**	IFG	42, 10, 12	*112*	**4.62**
SFG	16, 56, 40	*192*	**4.13**	SFG	20, 46, 16	*96*	**4.21**
SFG	−16, 36, 36	*88*	**3.82**	MFG	50, 52, −2	*96*	**4.20**
				MFG	−10, 64, −6	*104*	**4.01**
				SFG	18, 16, 62	*88*	**3.65**
				SFG	−4, 36, 32	*176*	**3.55**
Anxious symptoms (MASQ)							
Gyrus rectus	−2, 18, −30	*3.98*	**296**	MFG	34, 10, 58	*192*	**7.98**
				MFG	−36, 14, 44	*384*	**5.57**
				SFG	−16, 16, 50	*496*	**5.56**
				MFG	8, 36, −12	*176*	**5.49**
				SFG	6, 26, 70	*184*	**5.41**
				IFG	−28, 28, −18	*632*	**5.30**
				Putamen	26, 14, 14,	*320*	**4.22**
				MFG	−22, 26, 56	*96*	**4.14**
				ACC	−10, 22, 36	*304*	**4.07**
				IFG	54, 22, −10	*136*	**3.82**
				SFG	−6, 28, 68	*304*	**3.71**

Table 3 (*continued*)

Positive correlation				Negative correlation			
Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*	Brain area	MNI (*x*, *y*, *z*)	mm^3	*T*
				IFG	40, 14, 22	*136*	**3.71**
				MFG	28, 52, 32	*312*	**3.64**
Anhedonic symptoms (MASQ)							
MFG	18, 50, −20	*1072*	**6.73**	IFG	−52, 28, 12	*536*	**4.64**
MFG	28, 42, −12	*1056*	**6.29**	IFG	−56, 14, 26	*136*	**3.77**
SFG	8, 20, 54	*248*	**5.00**				
MFG	−30, 14, 62	*496*	**4.96**				
ACC	−14, 46, −6	*472*	**4.96**				
MFG	−30, 16, 46	*512*	**4.83**				
SFG	−14, 62, −8	*192*	**4.60**				
MFG	−24, 40, 24	*88*	**3.97**				
SFG	22, 62, 2	*464*	**3.84**				
Insula	34, 18, 14	*104*	**3.75**				
SFG	−24, 64, 2	*88*	**3.59**				

Analysis was restricted to prefrontal areas ($y \geqslant 9$). Data are shown for $p \leqslant 0.005$ uncorrected. All clusters are reported for $k > 10$ voxels.

associated with social withdrawal behavior as well as elevated basal and reactive cortisol levels. Our finding which indicates a positive association between a pattern of increased glucose metabolic rate in the right prefrontal cortex is also consistent with animal data showing that lesions in the right but not in the left PFC caused marked decreases in corticosterone in acutely restrained animals (Sullivan and Gratton, 1999).

The reversed pattern for stress-induced glucose metabolic rate and saliva cortisol concentrations we observed for BA 10 in particular is consistent with previous evidence indicating that BA 10 is an important site for voluntary regulation of negative emotion (Urry et al., 2006) and that activation of this region during down-regulation of negative affect was associated with lower basal evening cortisol levels, a steeper diurnal cortisol slope and reduced amygdala activation. In line with this finding, recent theories of medial prefrontal cortex function suggest that this region is involved in coordination of information processing and information transfer when multiple processes are engaged in the service of a behavioral goal (Ramnani and Owen, 2004), and in coordinating attention between external stimuli and internal thoughts (Burgess et al., 2005). There is also increasing evidence that the mPFC is involved in processes associated with social information processing (Gallagher and Frith, 2003; Iacoboni et al., 2004) and self-referential activity (Johnson et al., 2002). These findings, along with the functions ascribed to the mPFC in recent theoretical accounts (e.g. Ramnani and Owen, 2004) match those required to regulate negative affect during a socially threatening and highly uncontrollable task such as the TSST, since multiple streams of internal and external information processing would require coordination, including maintaining representations of the behavioral goal to decrease negative affect, monitoring and modifying spontaneous appraisals arising in response to stressor, and evaluating behavioral and social success.

Similar to BA 10, BA 9 reflects another important site when it comes to voluntary down-regulation of negative affective states (Levesque et al., 2003) and seems to be de-activated in the face of perceived negative emotions (sadness) (Liotti et al., 2000). In a recent study looking at the role of HPA axis activity on cognitive and emotional information processing of traumatic stimuli, pronounced covariation between basal as well as trauma-related plasma adrenocorticoid levels were found in BA 9 as well as BA 10 (Liberzon et al., 2007), indicating that these regions might be important for regulating stressful or traumatic information. Interestingly, medial aspects of BA 9 are anatomically connected with lateral columns of the periaqueductal grey (PAG) (An et al., 1998) and the lateral PAG has previously been associated with active emotional coping styles in response to stressor confrontation (Keay and Bandler, 2001). BA 9 could therefore serve as an integrative site which regulates affective states and corresponding active coping behavior during stressful instances.

Data presented here are correlational in nature and therefore do not permit inference regarding directional functionality. Thus the question remains, whether the PFC actually exerts causal inhibitory and excitatory effects over the HPA axis function in humans. Support for this hypothesis comes from animal findings, showing that lesions in dorsal mPFC regions result in an exaggerated HPA axis activity in response to stress exposure and these effects are reversed by implanting corticosterone pellets in the lesion site (Diorio et al., 1993). These findings further indicate that these inhibitory effects are actually mediated by glucocorticoids by binding to glucocorticoid receptors in medial prefrontal regions. Keeping in mind that data presented here reflect brain activity monitored over the course of a 25 min stress task, the effects seen here could also reflect some form of glucocorticoid based regulatory mechanism rather than glucocorticoid-independent short-term information processing of stress-relevant information. This interpretation could

also explain why activation in BA 9 and BA 10 was not reported in a recent study involving a stressor that was not only significantly shorter in duration but also resulted in much lower stress-induced cortisol increase (Wang et al., 2005).

Similarly, a recent study using imaging techniques with more precise time resolution (functional MRI and [0–15]–H20 PET), reported opposite findings of pronounced deactivations in limbic and prefrontal regions in the face of moderately increased cortisol levels (Pruessner et al., 2008). Thus, future studies on neural substrates of stress need to be very specific about the neural process of interest (e.g. fast initial information processing vs. delayed feedback mechanisms) and imaging techniques with an adequate time resolution should be applied.

The idea that the PFC serves as an entity that regulates internal affective states during instance of psychosocial stress is further supported by the finding that dispositional mood states such as general distress or depressive symptoms were associated with the endocrine stress response as well as distinct stress-induced prefrontal activation patterns. These data give room to speculate that the prefrontal cortex, depending on the exact location might be involved in translating emotional disposition into endocrine response patterns, which is in accordance with findings showing an association between mood disorders and endocrine dysregulations (Gillespie and Nemeroff, 2005). Interestingly, associations between general distress and depressive symptoms were most prominent in brain regions that have previously been associated with morphological and functional abnormalities in mood disorders (Rajkowska et al., 1999; Seminowicz et al., 2004).

When functional connectivity analyses were conducted, we observed that individuals with the greatest increases during the stress vs. control condition in the mPFC regions BA 9 and BA 10 had the smallest increases during these conditions in the amygdala/hippocampus, the precuneus, the fusiform gyrus, the inferior orbitofrontal gyrus and the medial temporal gyrus. While all these findings are correlational in nature and therefore do not permit inferences about causality, data presented are also consistent with the notion that activity in the mPFC may exert inhibitory effects on HPA function through its modulation of activation elsewhere in the brain, particularly in the amygdala/hippocampus, in light of what is known about the participation of these regions in anxiety and stress (Davis and Whalen, 2001).

This hypothesis is supported by rodent data, which implicate a central role of the mPFC in extinction of aversive learning with lesions of this region resulting in impairments of extinction (Milad and Quirk, 2002). Quirk et al. (2003) have also demonstrated that stimulation of mPFC in rats results in decreased responsiveness of output neurons in the central nucleus of the amygdala. Accordingly, in humans, increased BOLD signal in prefrontal regions has been associated with decreased amygdala activity in response to emotionally loaded stimuli (Hariri et al., 2003; Urry et al., 2006).

Not only the amygdala complex but also medial parietal regions (precuneus), the fusifrom gyrus as well as temporal and orbitofrontal areas have previously been associated with social cognition and information processing of emotionally and socially loaded information (Dalton et al., 2005; Iacoboni et al., 2004; Rojas et al., 2006). Findings from this connectivity analysis therefore give room to speculate that during a psychosocial stress exposure, the PFC modulates HPA activation in concert with a series of brain areas relevant for social and affective information processing.

A limitation of this study is the lack of FDG blood concentration measures and subsequent lack of quantitative PET data. Arterial or venous blood sampling and subsequent testing for tracer glucose concentration in arterial or arterialized blood allows for a kinetic model-based quantification of cerebral glucose metabolism. Globally normalized and grand-mean-scaled data as presented here do not permit quantification of cerebral glucose metabolism. Grand mean scaling and global normalization are significantly less invasive but also less conclusive when compared to quantitative data. However, even though quantification would permit a more conclusive analysis, the required blood sampling would have interfered with the fundamental psychosocial object of our experimental design.

Another limitation comes from the rather liberal statistical threshold applied for the PET data analysis. Using a liberal statistical threshold (e.g. not correcting for multiple comparisons) increases the risk of false positive results which is a serious concern. Quite regularly, associations between behavioral or physiological measures and brain activity are small in magnitude and restricted to circumscribed areas. Such associations are therefore often hard to detect in the presence of rigorous statistical procedures. One way to overcome this obstacle is to restrict statistical analysis to *a priori* defined regions of interest and to control for multiple comparisons within this defined space. However, in the absence of such locally restricted *a priori* hypothesis, more liberal statistical thresholds have previously been used to detect neural substrates of behavioral or physiological measures in larger areas of the brain (Kato et al., 2007; Perneczky et al., 2007). In this exploratory study, we were most interested in the role of the PFC in human HPA axis regulation during a psychosocial stress. In order to explore the contribution of various PFC regions in HPA axis regulation (e.g. medial vs. lateral vs. dorsal), we explicitly refrained form further specifying *a priori* regions of interest within the PFC. In order to address concerns regarding false positive results in the face of the above mentioned limitations, we restricted our initial analysis to prefrontal regions and a statistical threshold of $p = 0.005$ was applied for this analysis.

Finally, only male participants were considered in our study design. It is well known that stress-related saliva cortisol concentrations are significantly influenced by menstrual cycle phases or the use of oral contraceptives (Kirschbaum et al., 1999). This was regarded as a major confounding factor in this initial study and we therefore restricted our study design to male participants only. However, given what is known about sex-specific gender difference in neural processing of emotional information, our results might be specific to males. Future studies should involve female subjects, in order to elucidate possible gender difference in neural responses to emotionally loaded stress.

In summary, our data provide the first evidence that distinct areas within the prefrontal cortex show positive as

well as negative associations with endocrine stress measures and that these patterns are behaviorally associated with subjective ratings on task stressfulness, controllability and dispositional mood states. The data also highlight the fact that in response to a psychosocial stressor, large individual differences are present and such individual differences are associated with lawful patterns of neural activity. Future studies might target these individual differences as risk factors for the development of stress-related physical and psychiatric disorders.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2008.01.010.

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