Cortisol Administration Increases Hippocampal Activation to Infant Crying in Males Depending on Childhood Neglect

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Abstract: Animal studies show that exposure to parental neglect alters stress regulation and can lead to neural hyposensitivity or hypersensitivity in response to cortisol, most pronounced in the hippocampus. Cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, has also been related to parenting more directly, for example, in both sexes, cortisol levels increase when listening to infants crying, possibly to activate and facilitate effective care behavior. Severe trauma is known to negatively affect the HPA-axis in humans; however, it is unknown whether normal variation in parental care in the healthy population can alter sensitivity of the hippocampus to cortisol. Here, we investigate whether variation in experienced neglect changes neural sensitivity to cortisol when humans listen to infant crying, which is an unequivocal signal relevant for care behavior. In a placebo-controlled, within-subject neuroimaging study, we administered 40 mg cortisol to 21 healthy young males without children and used a validated task for measuring neural responses to infant crying. The Dutch version of the Childhood Trauma Questionnaire was used to index participants' early exposure to abuse and neglect. The data show that cortisol markedly increased hippocampal activation toward crying infants, and this effect varied significantly with parental neglect, even in our nonclinical subject sample. Without exposure to severe trauma or neglect, reduced self-experienced quality of parental care in the normal range already substantially increased hippocampal responsivity to cortisol. Altered hippocampal sensitivity to cortisol might be a cross-species marker for the risk of developing later life psychopathology. *Hum Brain Mapp* 35:5116–5126, 2014. © 2014 Wiley Periodicals, Inc.

Key words: fMRI; steroid hormones; parental behavior; childhood trauma

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INTRODUCTION

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It is well established that poor parental care in childhood such as neglect and maltreatment brings increased risk for later life psychopathology, as well as for poor parental care toward one's own children later in life [Lanius et al., 2010; Meaney, 2001]. Given the major impact on society, research investigating the neural pathways by which adverse childhood experiences have such debilitating effects later in life is of utmost importance [Anda et al., 2010; Shonkoff et al., 2009]. An important mechanism proposed to underlie the effects of early life stress, such as neglect, is altered neural sensitivity for cortisol

(corticosterone in rats, from here on both referred to as CORT), the end product of the hypothalamic-pituitary-adrenal (HPA) stress axis [Heim et al., 2008; Lupien et al., 2009; Meaney, 2001]. This altered sensitivity can result in a hypoactive or hyperactive stress system, which can have detrimental effects on typical brain development, thereby interfering with parental care later in life [Champagne, 2011; Heim et al., 2008; Pechtel and Pizzagalli, 2011]. CORT has also been linked to parenting more directly, as fathers show increases in CORT when listening toward crying infants, allegedly to facilitate adequate responding to the child's needs [Fleming et al., 2002]. As such, experienced neglects in childhood can alter an individual's sensitivity to CORT and can thereby affect the neural responses to crying, the infant's most elementary social signal.

In animals, the effects of differences in parental care on neural sensitivity for CORT have been thoroughly investigated. The amygdala and especially the hippocampus are sensitive to alterations in parental care, and severity of neglect powerfully moderates these effects [Champagne, 2011; Heim et al., 2008; Lupien et al., 2009; Meaney, 2001]. Both regions are also densely distributed with CORT receptors, and of these regions, the hippocampus is crucially involved in the downregulation of the HPA-axis in the aftermath of stress [Jacobson and Sapolsky, 1991; McEwen, 2010]. Severe neglect induced by maternal separation, whereby an infant rat is removed from the mother several hours a day, has detrimental effects on the HPA-axis. It results in reduced CORT-receptor expression in the hippocampus and also in increased basal and stress-induced CORT levels in adulthood [Lupien et al., 2009; Oitzl et al., 2010]. Reduced sensitivity of the hippocampus to CORT after severe neglect can thus result in less feedback to attenuate the HPA-axis, causing altered levels of stress hormones, and subsequently interferes with normal brain development. In contrast to the detrimental effects of severe neglect, studies in rats subjected to handling (short periods of separation from the mother) show an opposite pattern of effects on the stress system, that is, reduced basal and stress-induced CORT levels, as well as less anxious behavior [Meaney, 2001; Oitzl et al., 2010]. Thus, depending on the severity of neglect, hippocampal sensitivity to CORT can either increase or decrease.

In humans, severe early life adversity disrupts normal functioning of the HPA-axis and leads to structural changes of the hippocampus and amygdala [Andersen and Teicher, 2009; Heim et al., 2008; Lanius et al., 2010; Lupien et al., 2009]. The neuroendocrine and behavioral effects of severe neglect, which are mostly investigated in individuals with later psychopathology, have received substantial attention; however, much less attention is directed to effects of normal variation in healthy humans [Lanius et al., 2010]. The neural effects of CORT administration in healthy humans have often been investigated in the context of memory formation [e.g., Henckens et al., 2012a; Oei et al., 2007] and face processing [e.g., Henckens et al., 2010]. These studies have generally shown amygdala

and hippocampal deactivation and decoupling [Henckens et al., 2012b] after CORT administration. However, measures of parental care and neglect were not taken into account in these studies, and it is thus unknown whether variation in parental neglect alters neural sensitivity to CORT. If so, such altered neural sensitivity could subsequently affect parenting behavior in humans, comparable with the mechanisms observed in rodents [Meaney, 2001; Oitzl et al., 2010]. A similar mechanism was proposed for the effects of harsh parenting on sensitivity to oxytocin [Bakermans-Kranenburg and Van IJzendoorn, 2013; Riem et al., 2013], a key hormone in parental bonding [Rilling, 2013].

Importantly, CORT has also been linked to parenting in both human males and females more directly. Higher CORT levels in mothers predict increased sensitivity for infant cues, a more positive attitude toward their own children [Fleming et al., 1997] and also more affectionate responses toward infant crying [Stallings et al., 2001]. In fathers, CORT rises in response to crying, provisionally to deal with such a social challenge [Fleming et al., 2002]. Infant crying also elicits neural activation in the thalamus, insula, amygdala, and hippocampal regions in mothers and fathers [Swain et al., 2007]. Of these regions, specifically activation of the hippocampus correlates with the level of maternal bonding [Musser et al., 2012]. Based on these correlational findings, the sensitivity of the amygdala, and especially of the hippocampus to variation in adverse childhood experiences, we hypothesize that variation in parental neglect will alter neural sensitivity in these regions to CORT in response to infant crying in humans.

To test this hypothesis, CORT was administered to a group of young healthy males without children, who filled out a validated questionnaire that measures childhood neglect [Thombs et al., 2009]. Functional magnetic resonance imaging (fMRI) was used to measure neural activation when the subjects were exposed to infant crying and control sounds. In addition to whole-brain analyses, region of interest analyses were performed on the bilateral hippocampus, insula, amygdala, and thalamus.

MATERIALS AND METHODS

Subjects

Twenty-one healthy, right-handed males without children (mean age = 22.8 years, SD = 2.72) participated in this study, which was approved by the Medical Ethical committee of the University Medical Centre Utrecht and in accordance with the declaration of Helsinki. Only males were included in the current study as males respond differently to CORT administration when compared with females [e.g., Merz et al., 2010]. In addition, there are also indications that males are more susceptible to the neural and behavioral effects of childhood neglect [De Bellis, 2010; McCullough et al., 2013], and we therefore focused

exclusively on males in the current study. Participants were screened for physical or mental illness, abuse of alcohol and drugs, and regular smoking, all of which were exclusion criteria. Participants earned a standard reimbursement of 60 euros with an additional amount that could be obtained in a monetary task which was also performed during the scan session (will be reported elsewhere). Because of the technical issues, the data of this monetary task were lost for one participant which was therefore replaced. As the data of the cry task were not lost, this resulted in an additional participant to the 20 participants planned.

Drug Administration and Procedure

The current experiment followed a placebo-controlled, counterbalanced within-subjects CORT administration setup. The experiment always took place between 02.00 and 9.00 p.m., when CORT levels are relatively low. To control for diurnal fluctuations of CORT, the time of the day on which testing took place was kept similar within subjects. Subjects arrived at the laboratory to fill out the Childhood Trauma Questionnaire Short Form (CTQ-SF) and provided a saliva sample by passively drooling in a plastic vial to obtain baseline CORT levels. Subsequently, the drug was administered under supervision of the experimenter. CORT was orally administered in a capsule, containing 40 mg hydrocortisone + 320 mg Primogel, whereas the placebo consisted of only 360 mg Primogel. About 45-min postdrug intake, subjects were placed in the MRI scanner after which an anatomical scan was made. After the first task (monetary task; will be published elsewhere), the cry task started at a constant interval of 60 min after the administration. The dosage of CORT and the drug-testing interval that we used are comparable with earlier studies that were conducted in our laboratory [for a review, see Montoya et al., 2012]. Similar dosages (30 and 35 mg) using the same time-testing interval (~60 min) have also been used by others, showing behavioral and physiological effects of CORT administration [e.g., Merz et al., 2010; Oei et al., 2009]. After the scan session, subjects were asked to rate how intense, emotional, natural, and annoying the crying and control stimuli were perceived on a visual analog scale, which ranged from -100 to 100. After the second session, participants were debriefed and given payment.

Mood and Hormone Measurements

Saliva samples (5 ml) were collected before drug intake and were stored in a freezer at -20°C until assayed. Laboratory analyses were performed at the Department of Biopsychology, TU Dresden, Germany. Saliva samples were thawed and spun at 3,000 rpm for 10 min to obtain clear supernatant with low viscosity. Salivary CORT concentrations were determined by a commercially available

chemiluminescence immunoassay (IBL Hamburg, Germany). Subjects were asked to refrain from eating 1 h before providing the saliva sample. Mood state was measured with the Profile of Mood States (POMS) questionnaire [Shacham, 1983], before and after drug intake to investigate if the CORT administration resulted in altered mood.

Cry Task and Questionnaire

The cry task used in the current study was also used in a previous study and consists of a block design task including three conditions: infant crying, control sound, and a control condition wherein no sound was presented [Bos et al., 2010]. Block duration was 25 s, with six blocks of each condition. Crying sounds of infants ranging from 1 to 5 months (www.audionetworkplc.com) and were adapted to create six different sound files with durations of 25 s. Control sound files were created using Matlab 7.5 (The Mathworks, Natick, MA) by segmenting the cry stimuli into 5-ms sound files, which were randomized and reassembled. Afterward, the control files were overlaid with the volume intensity modulations of the cry stimuli. This led to control sounds no longer recognizable as crying, but with comparable volume and audio spectral properties. To control whether participants attended to the stimuli throughout the experiment, nine sine-wave tones (1 kHz, 100 ms) were presented in response to which participants were instructed to press a button. These sine-wave tones were pseudo-randomly presented; in every condition, three tones were presented, and never twice during a single stimulus presentation. Auditory stimuli were presented using an electrodynamic headphone system (Confon HP-SI01; Leibniz-Institute for Neurobiology, Magdeburg, Germany). Participants were given a MRI-compatible buttonbox and were instructed to attend to the sounds they would hear.

The Dutch version of the CTQ-SF [Thombs et al., 2009] was used to assess childhood neglect and maltreatment. The CTQ-SF consists of the subscales emotional abuse, physical abuse, sexual abuse, and emotional neglect and physical neglect, of which the items are rated on a five-point scale ranging from "never true" to "very often true." The scores on all subscales of the CTQ-SF in the current study were comparable with those obtained in the nonclinical sample of a validation study [Thombs et al., 2009]. According to the cutoff scores reported in the CTQ-SF manual [Bernstein and Fink, 1998], scores of 18 participants were in the "none-to-low" category on all subscales, and three participants scored in the "moderate" category on one of the subscales. The averages in the current sample are as follows: emotional abuse, 6.8; physical abuse, 5.4; sexual abuse, 5.0; emotional neglect, 10.1; and physical neglect, 6.2.

fMRI Scanning Parameters and Analysis

Scanning was performed on a 3 Tesla Philips Achieva MRI scanner (Philips Medical Systems, Best, The

TABLE I. Peak T-values, P-values, cluster sizes, and MNI coordinates for significantly activated voxels

Experimental effect and region		Peak voxel location			Cluster size	T-values	P-values
		x	y	z			
Full factorial:					Voxels		
Main effect: sound > rest							
Superior temporal cortex	R	60	-22	2	3,733	15.28	<0.001 ^a
	L	-46	-16	-2	3,660	13.20	<0.001 ^a
Insula	R	42	-20	-20	s.c.	13.45	<0.001 ^a
	L	-44	-18	-2	s.c.	12.62	<0.001 ^a
Thalamus	L	-26	-30	10	s.c.	7.27	<0.001 ^a
	R	16	-26	-8	2	5.06	0.022 ^a
Hippocampus	L	-38	-18	-14	s.c.	5.60	0.003 ^a
	R	38	-18	-12	s.c.	5.14	0.016 ^a
	R	16	-28	-10	1	4.89	0.035 ^a
Lingual gyrus	R	14	-28	-8	19	5.09	0.018 ^a
Amygdala	R	38	-4	-20	18	4.43	0.004 ^b
	R	26	-8	-16	46	3.63	0.031 ^b
	L	-22	-4	-20	187	4.33	0.004 ^b
Main effect: cry > control							
Superior temporal cortex	R	62	-20	-4	46	5.56	0.004 ^a
	R	60	-2	-6	5	4.89	0.035 ^a
Main effect: control > cry							
Adjacent to inferior temporal cortex	L	-40	-44	-6	54	5.38	0.007 ^a
Adjacent to Heschl's gyrus	L	-36	-22	-4	13	5.10	0.018 ^a
Insula	L	-38	-22	-4	s.c.	5.07	0.022 ^a
	R	38	-18	-4	78	4.24	0.005 ^b
Interaction: cortisol (cry > control) × placebo (control > cry)							
Hippocampus	R	32	-34	-2	37	4.59	0.004 ^b
	L	-16	-28	-18	28	3.98	0.025 ^b
	L	-24	-36	6	8	3.77	0.046 ^b
Thalamus	L	-24	-32	8	36	4.33	0.008 ^b
Simple effects placebo condition:							
Cry > morph							
Superior temporal cortex	R	62	-20	-6	61	5.60	0.003 ^a
Morph > cry							
Adjacent to inferior temporal cortex	L	-40	-44	-6	26	5.58	0.003 ^a
Insula	R	40	-18	-2	50	3.87	0.015 ^b
	L	-38	-20	-6	29	3.87	0.015 ^b
	L	-36	-18	10	38	3.70	0.024 ^b
Thalamus	L	28	-24	-4	24	3.96	0.023 ^b
	R	-20	-32	-4	20	3.83	0.033 ^b
Hippocampus	L	-24	-30	-6	33	3.96	0.026 ^b
Simple effects cortisol condition:							
Cry > morph							
Hippocampus	L	-16	-30	-20	20	4.27	0.010 ^b
Morph > cry							
Insula	L	-36	-22	-2	23	3.75	0.021 ^b

Abbreviations: R, right; L, left; s.c., same cluster as above.

^aWhole-brain FWE corrected (peak level).

^bSmall volume FWE corrected (peak level).

Netherlands). Before the functional scans, a high-resolution anatomical T1-weighted scan with the following parameters was obtained for coregistration and normalization purposes: 3.8 ms echo time, 8.4 ms repetition time, 288 mm × 288 mm × 175 mm field of view, 175 sagittal slices, flip angle of 8.0°, and voxel size 1.0 mm isotropic. Blood oxygen level-dependent response was

measured with functional T2*-weighted axial whole-brain images, of which 323 were obtained throughout the task. The 2D EPI-SENSE sequence had the following parameters: echo time 23 ms, repetition time 1.4 s, 208 mm × 119 mm × 256 mm field of view, 30 slices, flip angle of 70°, voxel size 4.0 mm isotropic, and SENSE-factor $R = 2.4$ (anterior–posterior).

Figure 1.

Axial slices with accompanying MNI coordinates for the Z-axis of the *t*-maps for the contrasts of (A) both sound conditions versus rest and (B) cry versus control sound (plotted in white-to-red color spectrum) and for control sound versus cry (plotted in blue color spectrum). *t*-maps are overlaid onto a T1-weighted canonical image and thresholded at $P < 0.001$ uncorrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Preprocessing and subsequent analyses were performed with SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>). Functional scans of both sessions were motion corrected to the first dynamic scan and slice-time corrected to the middle slice. The anatomical scan was then coregistered to the mean functional scan. Subsequently, using unified segmentation, the structural scan was segmented, and the normalization parameters were estimated. Using these normalization parameters, all volumes were normalized to a standard brain template (MNI) and were resliced at 2 mm isotropic voxel size. Smoothing with an 8 mm full width at half maximum Gaussian kernel was applied to the normalized functional volumes.

A general linear model was applied to the data to investigate the main effects of stimulus (effect of the cry sounds) and its interaction with CORT administration. Within both sessions, neural responses to exposure of the cry sound and the morph sound are modeled using a 25-s

boxcar function convolved with a hemodynamic response function (hrf) as implemented in the SPM8 software. Additional regressors of no interest that are entered into the analyses to reduce unexplained variance in the data include the realignment parameters, a discrete cosine transform high-pass filter with a cutoff of 128 s, and a hrf-convolved onset of the sine-wave tone to which participants had to respond. The contrast maps of cry versus baseline and control sound versus baseline in both sessions were entered in a second-level factorial ANOVA, with drug (CORT vs. placebo) and stimulus (cry vs. control sound) as within-subject factors and order as a between-subject factor.

For all calculated linear contrasts, the statistical threshold was set at $P < 0.05$ Family Wise Error (FWE) corrected for peak level. For display purposes only, a $P < 0.001$ uncorrected threshold was applied. In addition to the whole-brain analyses, the following regions of interest were defined: hippocampus, insula, amygdala, and thalamus. Small volume corrections with the same statistical threshold of $P < 0.05$ FWE were applied to anatomically defined bilateral masks based on the probabilistic cytoarchitectonic maps [Amunts et al., 2005] of the Anatomy toolbox as implemented in the SPM8 software [Eickhoff et al., 2007]. To further analyze the effects of CORT on the hippocampus, we extracted percent signal change within the left and right hippocampus using MarsBaR [Brett et al., 2002]. Using anatomically defined masks (Anatomy toolbox) [Eickhoff et al., 2007], circularity in this analysis is avoided [Kriegeskorte et al., 2009]. The output values from MarsBaR for both the left and right hippocampus were subsequently entered in a 2X2 multivariate ANOVA with drug administration (CORT vs. placebo) and stimulus conditions (cry vs. control sound) as separate factors. Order was entered as a between-subjects factor and was removed from the analyses if it showed not significant. Post hoc comparative *t*-tests were performed to further specify the effects.

Finally, to test our hypothesis whether effects of CORT on the hippocampus are mediated by childhood neglect, we calculated whole-brain contrast maps for cry versus control sounds in both the CORT and placebo condition and added the neglect subscales of the CTQ-SF as a covariate in separate second-level analysis to investigate the effect of these measures on the increased hippocampal activation to crying. To visualize the observed effects, we additionally correlated the neglect subscales of the CTQ-SF with the difference score between cry and control sounds in both the CORT and placebo condition.

RESULTS

Imaging Data

The main contrast of both sound conditions versus rest yielded large clusters of activation in the bilateral temporal cortex, of which the peak voxels were located in the

Figure 2.

A: Sagittal slices with accompanying MNI coordinates for the X-axis of the t-maps for the interaction between drug condition and sound condition cortisol. The t-map is thresholded at $P < 0.001$ uncorrected and is overlaid onto a T1-weighted canonical image, which shows the anatomically defined bilateral hippocampi in green. **B:** Bar graph of the extracted values from the bilateral hippocampi. The error bars represent standard error of the mean. There was an overall significant interaction between stimulus type and cortisol administration [$F(1,20) = 5.02$; $P = 0.037$, $\eta^2_p = 0.20$]. Post hoc *t*-test revealed a significant difference between responses toward crying in the placebo and cortisol condition in the left side [$t(20) = 2.61$; $P = 0.017$, $d = 0.74$], whereas in response to the control sounds, cortisol had no such effect [$t(20) = 0.42$; $P = 0.68$, $d = 0.12$]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

superior temporal cortex (Table I and Fig. 1A). The clusters extended into the thalamus, hippocampus, insula, and the primary and secondary auditory cortices: Heschl's gyrus, rolandic operculum, and medial and superior temporal cortex, a pattern of activation comparable with that previously observed using the same task in women [Bos et al., 2010]. The main effect of crying versus control sound activated the anterior part of the right superior temporal cortex, which corresponds to the role of this region in processing complex auditory information such as human speech [Rauschecker and Scott, 2009]. The opposite contrast of the control sound versus the cry sound resulted in bilateral activation of the primary auditory regions:

Heschl's gyrus and rolandic operculum, which extended into the bilateral insula (Fig. 1B).

Central to the predictions in the current study are the effects of CORT on the observed neural activation. Although there was no main effect of CORT on both sound stimuli, there was a significant interaction between CORT administration and sound type. Crucially, after CORT administration, the bilateral hippocampus responded more strongly toward cry sounds when compared with the opposite contrast in the placebo condition (Fig. 2A and Table I), and this activation was located in the posterior part of the hippocampus [Chen and Etkin, 2013]. In addition to the bilateral hippocampus, we also observed activation of the left thalamus for this contrast. In the opposite interaction (i.e., more activation for placebo cry versus control sound when compared with the opposite contrast in the CORT condition), no effects were observed. In general, linear contrasts other than those reported here did not show activation that survived the statistical threshold of $P < 0.05$ FWE corrected; furthermore, we did not observe an effect of administration order in the data.

Next, we investigated this interaction by testing the effect of cry versus control sounds in the placebo and CORT condition separately (Table I). These analyses showed that the effects in the placebo condition were similar to those of the overall analysis of both sessions reported above, whereas only in the CORT condition, a significant effect of the cry stimuli in the left hippocampus was observed. To further analyze this effect of CORT on the hippocampus, we extracted the percent signal change for the anatomically defined hippocampus (Fig. 2B). Overall, there was a significant interaction between stimulus type and CORT administration [$F(1,20) = 5.02$; $P = 0.037$, $\eta^2_p = 0.20$], which was significant in the left hippocampus [$F(1,20) = 6.04$; $P = 0.023$, $\eta^2_p = 0.23$] but not in the right hippocampus [$F(1,20) = 3.63$; $P = 0.071$, $\eta^2_p = 0.15$]. In line with the whole-brain analyses, post hoc *t*-test revealed that in the placebo condition, the left hippocampus responded more strongly to the control sound when compared with the cry sound [$t(20) = -3.03$; $P = 0.007$, $d = 0.47$], whereas after CORT administration, no such effect was observed [$t(20) = 0.53$; $P = 0.60$, $d = 0.15$]. The difference between hippocampal responses toward crying in the placebo and CORT condition was significant in the left hippocampus [$t(20) = 2.61$; $P = 0.017$, $d = 0.74$], whereas in response to the control sounds, CORT administration had no such effect [$t(20) = 0.42$; $P = 0.68$, $d = 0.12$]. In summary, CORT increased left hippocampal activation selectively toward sounds of crying infants, whereas there was no effect of CORT in response to control sounds.

Finally, we predicted that effects of CORT on the hippocampus would be modulated by experienced childhood neglect. To test this hypothesis, we investigated the effect of the neglect subscales of the CTQ-SF on the increased hippocampal activation to crying in both the CORT and placebo condition in whole-brain analyses. In these

TABLE II. Peak T-values, P-values, cluster sizes, and MNI coordinates for significantly activated voxels

Experimental effect		Peak voxel location			Cluster size	<i>T</i> -values	<i>P</i> -values
Region		<i>x</i>	<i>y</i>	<i>z</i>	Voxels		
Interaction cortisol condition:							
Cry > morph × emotional neglect scale							
Hippocampus	L	−24	−36	0	129	5.40	0.005 ^a
Cry > morph × physical neglect scale							
Hippocampus	L	−26	−34	0	49	4.43	0.030 ^a

Abbreviations: L, left.

^asmall volume FWE corrected.

analyses, both the emotional and physical neglect scales showed significant in the left hippocampus (Table II and Fig. 3A) in the CORT condition but not in the placebo condition. To visualize and further analyze the effect of neglect on the left hippocampus, we computed the difference between cry and control sound, for the extracted values of the left hippocampus in the CORT and placebo condition, and correlated these measures with the subscales of the CTQ-SF. As normality of the subscale scores could not be assumed (Shapiro-Wilk test, $P > 0.05$), Spearman's rank order correlation coefficients were calculated. This analysis confirmed the whole-brain analysis, by showing a significant positive correlation of the emotional neglect scale with the activation of cry sound versus control sound after CORT ($\rho = 0.59$; $P = 0.006$; Fig. 3B), although the physical neglect scale did not reach significance ($\rho = 0.39$; $P = 0.08$). In the placebo condition, no significant correlations were found (both $\rho < 0.18$; $P > 0.41$). In summary, the emotional and physical neglect scale both predicted the effect of CORT on the left hippocampus, in which the more neglect our participants experienced, the stronger the hippocampus responded to infant crying after CORT administration.

Behavioral Data

After both scan sessions, participants were asked to rate how intense, emotional, natural, and annoying the cry and control sounds were experienced, and these ratings were entered in a 2X2 multivariate ANOVA with drug administration and sound type as within-subject factors. The analysis was performed on 20 subjects, as the ratings of one subject were not available. There were no effects of drug or sound type on the intensity rating or on how annoying the sounds were experienced (all P -values > 0.19). There was an effect of sound type on how natural [$F(1,19) = 33.49$; $P < 0.001$] and on how emotional [$F(1,19) = 31.32$; $P < 0.001$] the stimuli were perceived. Irrespective of drug condition, the crying sounds were experienced as more emotional and more natural (averages: cry emotional = -34.4; control emotional = -83.75; cry natural = 22.85; control natural = -58.63). Additionally, the order of administration did not significantly affect the behavioral data in any way.

As a control for subjective effects, CORT did not affect mood state as measured by the POMS questionnaire [Shacham, 1983]. Comparisons between the placebo and CORT

Figure 3.

A: Sagittal slices with accompanying MNI coordinates for the X-axis of the t-maps for the interaction between the neglect subscales of the CTQ-SF, and the activation in response to crying in the CORT condition. The t-map is thresholded at $P < 0.001$ uncorrected and is overlaid onto a T1-weighted canonical image, which shows the anatomically defined bilateral hippocampi in green. **B:** Correlation between the physical neglect scale of the CTQ-SF, and the effect of cortisol on the left hippocampus (difference score placebo – cortisol in the cry condition). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

condition of the difference scores between the preintake and postintake measures on the POMS subscales were all nonsignificant (all P -values > 0.05). Participants did not correctly guess the day of CORT administration (binomial, $P > 0.05$), showing that the effects that we found of CORT cannot be attributed to alterations in mood, or to subjective effects of drug administration.

Hormone Measurements

The CORT baseline levels in both conditions were positively skewed (skewness = 1.99 and 1.26; both $P < 0.05$), and therefore log-transformed and averaged over both test days. To see whether CORT baseline had any effect on the data, we correlated this average with the behavioral ratings and the subscales of the CTQ-SF, and the log-transformed saliva levels of the placebo condition with extracted imaging data from the hippocampus. None of the correlations showed significance (all P -values > 0.05).

DISCUSSION

In a pharmacofMRI study using placebo-controlled CORT administration, we tested whether neglect in early childhood would alter neural sensitivity to CORT. A validated fMRI paradigm was used in which male subjects were exposed to social signals relevant for parenting behavior: sounds of crying infants [Bos et al., 2010; Sander et al., 2007; Seifritz et al., 2003; Swain et al., 2007]. Similar to previous studies in males [Sander et al., 2007; Seifritz et al., 2003; Swain et al., 2007], the task elicited strong overall activation in the bilateral auditory regions, the thalamus, the amygdala, and the hippocampus. The cry condition elicited selective activation of the superior temporal cortex, whereas the control sounds yielded stronger activation in Heschl's gyrus and the inferior temporal cortex, and this activation extended into the insula (Fig. 1B). This pattern of activation appears to reflect more primary auditory cortex activation by the control sounds, and more the secondary auditory cortices by the cry sounds, which corresponds to the role of these areas in the respectively encoding, and more elaborate processing of auditory information [Schirmer and Kotz, 2006]. In line with our expectations, the data show that CORT administration increases activation of the posterior hippocampus selectively toward infant crying and that this effect depends on childhood neglect (Fig. 3). Subjects who experienced more neglect showed the strongest increase in left hippocampal activation in response to infant crying after CORT. Thus, normal variation in physical and emotional neglect resulted in altered sensitivity of the hippocampus, as measured by fMRI, to CORT administration.

These effects of CORT on the hippocampus translate to the animal studies which show that depending on severity, experienced neglect can increase or decrease hippocampal sensitivity to CORT [Meaney, 2001; Oitzl et al.,

2010]. Perhaps a comparable effect is observed in the current data, in which the mild neglect experienced by our participants sensitized the hippocampus to CORT. Enhanced hippocampal sensitivity to CORT could lead to stronger downregulation of the HPA-axis [Jacobson and Sapolsky, 1991]. Such increased downregulation has indeed been found in post-traumatic stress disorder [Yehuda et al., 2006], and reduced CORT levels have been observed in both healthy and depressed individuals who experienced childhood neglect, as measured with the CTQ-SF [Hinkelmann et al., 2013]. Altered sensitivity to CORT after childhood neglect or trauma signals an abnormality in the function of the HPA-axis and increases the likelihood of developing psychopathology such as PTSD. Such a trajectory might also contribute to the reported effects of childhood experience and CORT reactivity on structural deviations of the hippocampus [e.g., Andersen and Teicher, 2009; De Bellis, 2010; Narita et al., 2012].

An important question with respect to the current data is how our increased hippocampal activation relates to other studies that mostly report decreased hippocampal activation after CORT [e.g., Henckens et al., 2012a; Oei et al., 2007]. As these studies investigated memory processes, it might be that the effects of CORT are task dependent, similar to the effects of other hormones such as oxytocin and testosterone [Bartz et al., 2011; Bos et al., 2012]. However, an important limitation of fMRI is that it omits a true baseline, and recent studies indicate that CORT administration leads to fast global deactivation of the thalamus and hippocampus when compared with placebo [Lovallo et al., 2010; Strelzyk et al., 2012]. Our effects might thus represent an increase of activation toward crying relative to an overall decrease of metabolism in the CORT condition when compared with placebo. Another question is that why our effects were observed in the posterior part of the hippocampus, and whether these effects are the direct effect of CORT or indirect effects of CORT acting elsewhere in the brain, as CORT can have brain-wide effects [de Kloet et al., 1999]. The posterior hippocampus is tightly connected to the anterior and posterior cingulate cortex (ACC/PCC), which are regions involved in environmental exploration [Fanselow and Dong, 2010] and also key regions in the mammalian neural circuitry for parental care and cry responsiveness [Newman, 2007]. However, the posterior hippocampus is also strongly connected to the anterior hippocampus, which is tightly linked to the amygdala [Fanselow and Dong, 2010]. These regions are all important targets of CORT, and although we did not find effects on these regions [in contrast to others, e.g., Henckens et al., 2010], we cannot exclude that CORT acted on the posterior hippocampus via these regions. Irrespective of drug condition, we did not find effects of the cry stimuli in the amygdala, which agrees with our findings using this crying paradigm in nonparental young women [Bos et al., 2010] and another finding with nonparental males [Seifritz et al., 2003].

A final question is which underlying neurobiological mechanism is responsible for the increased hippocampal

activation after CORT, as the pathways by which CORT can act on the brain are manifold and are time and dose dependent [Groeneweg et al., 2011; Popoli et al., 2012]. CORT can directly alter neurotransmission by acting on glucocorticoid and mineralocorticoid receptors (GR/MR) and also indirectly by increasing endocannabinoid production [Popoli et al., 2012]. Fast effects of CORT on the hippocampus and amygdala are established by rapid release of glutamate, and most likely involve nongenomic MR function [Groeneweg et al., 2011; Karst et al., 2005; Popoli et al., 2012]. Slower effects of CORT can be directly induced by genomic effects on nuclear GRs [Oitzl et al., 2010] and also indirectly by upregulation of endocannabinoids, resulting in inhibition of the release of glutamate and GABA [Popoli et al., 2012]. Although our time interval of 1 h does not exclude the relevance of slower pathways [Oitzl et al., 2010], a recent study which compared slow and fast effects of CORT only found a deactivation of the hippocampus after the long interval [Henckens et al., 2012a], which suggests that our effects unlikely involve slow range effects of CORT administration. Effects of CORT might also interact with oxytocin at the level of the amygdala [Struber et al., 2014]. Oxytocin can increase after listening to infant crying [Swain et al., 2011] and can subsequently decrease amygdala activation to such sound [Riem et al., 2011]. Oxytocin furthermore attenuates CORT responses to stress [Heinrichs et al., 2003], and neural sensitivity to oxytocin has importantly been shown to also depend on parental neglect [Bakermans-Kranenburg and Van IJzendoorn, 2013; Grimm et al., in press; Riem et al., 2013]. Thus, in response to infant crying, the effects of oxytocin might be antagonistic to the effects of CORT in the amygdala, which is an interesting avenue for further research.

In relation to these issues, a limitation of the current study is that we did not measure CORT levels directly after the task. As cry stimuli are known to elicit CORT responses in fathers [Fleming et al., 2002], this information would have allowed us to analyze whether such increases (in nonfathers) are also related to neural activation in response to crying. It is important for future studies to investigate this issue and to also include women to investigate sex differences in brain function, as these have been observed in relation to CORT after early life stress [Burghy et al., 2012].

In conclusion, we here show that exposure to neglect in nonclinical range results in stronger hippocampal responses toward crying infants after CORT, a signal highly relevant for caring behavior. This demonstrates that normal variation in experienced care in childhood, without exposure to severe trauma or neglect, already changes the neural sensitivity of the human brain and specifically the hippocampus to hormonal factors. These findings importantly contribute to our understanding of the neuroendocrinology underlying human parenting behavior, as increased CORT seems to be an adaptive response toward crying infants [Fleming et al., 2002], and hippocampal acti-

vation has been related to quality of maternal bonding [Musser et al., 2012]. Insight into the dynamics of these mechanisms are of major importance, because negative responses to infant crying by caregivers are a risk factor for child abuse [Crouch et al., 2008], and HPA alterations are thought to mediate the relationship between childhood adversity and later life aggression [Veenema, 2009].

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