Associations Among Parenting Experiences During Childhood and Adolescence, Hypothalamus-Pituitary-Adrenal Axis Hypoactivity, and Hippocampal Gray Matter Volume Reduction in Young Adults

Kosuke Narita,^{1,*} Kazuyuki Fujihara,¹ Yuichi Takei,¹ Masashi Suda,¹
Yoshiyuki Aoyama,¹ Toru Uehara,¹ Takehiko Majima,¹ Hirotaka Kosaka,²
Makoto Amanuma,³ Masato Fukuda,¹ and Masahiko Mikuni¹

¹Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine,
3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan

²Department of Neuropsychiatry, University of Fukui, Eiheiji, Fukui 910-1193, Japan

³Department of Diagnostic Radiology and Nuclear Medicine, Gunma University Graduate School of
Medicine, Gunma 371-8511, Japan

Abstract: Recent human studies have indicated that adverse parenting experiences during childhood and adolescence are associated with adulthood hypothalamus-pituitary-adrenal (HPA) axis hypoactivity. Chronic HPA axis hypoactivity inhibits hippocampal gray matter (GM) development, as shown by animal studies. However, associations among adverse parenting experiences during childhood and adolescence, HPA axis activity, and brain development, particularly hippocampal development, are insufficiently investigated in humans. In this voxel-based structural magnetic resonance imaging study, using a cross-sectional design, we examined the associations among the scores of parental bonding instrument (PBI; a self-report scale to rate the attitudes of parents during the first 16 years), cortisol response determined by the dexamethasone/corticotropin-releasing hormone test, and regional or total hippocampal GM volume in forty healthy young adults with the following features: aged between 18 and 35 years, no cortisol hypersecretion in response to the dexamethasone test, no history of traumatic events, or no past or current conditions of significant medical illness or neuropsychiatric disorders. As a result, parental overprotection scores significantly negatively correlated with cortisol response. Additionally, a significant positive association was found between cortisol response and total or regional hippocampal GM volume. No significant association was observed between PBI scores and total or regional hippocampal GM volume. In conclusion, statistical associations were found between parental

Additional Supporting Information may be found in the online version of this article.

Clinical registration: The trial name—Examination regarding hypothalamus-pituitary-adrenal axis activity in mood disorder and post-traumatic stress disorder (PTSD) as determined by dexamethasone/corticotropin releasing hormone (DEX/CRH) test.

Contract grant sponsor: Japan Society for the Promotion of Science (JSPS); Contract grant numbers: 18790826 and 21791110; Contract grant sponsor: Mitsubishi Pharma Research Foundation.

*Correspondence to: Kosuke Narita, Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. E-mail: knarita@med.gunma-u.ac.jp

Received for publication 15 September 2010; Revised 27 March 2011; Accepted 18 April 2011

DOI: 10.1002/hbm.21354

Published online 3 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

overprotection during childhood and adolescence and adulthood HPA axis hypoactivity, and between HPA axis hypoactivity and hippocampal GM volume reduction in healthy young adults, but no significant relationship was observed between any PBI scores and adulthood hippocampal GM volume. *Hum Brain Mapp* 33:2211–2223, 2012. © 2011 Wiley Periodicals, Inc.

Key words: Hippocampus; parenting; dexamethasone; corticotropin-releasing hormone; magnetic resonance imaging

INTRODUCTION

The hypothalamus-pituitary-adrenal (HPA) axis activity, a major factor contributing to the enhancement or attenuation of cortisol release, has been considered as a potential marker of vulnerability to several stress-related diseases and neuropsychiatric disorders [Claes, 2004]. A reduced HPA axis activity, i.e., an attenuated cortisol response to acute psychosocial stress or neuroendocrine challenge, is associated with post-traumatic stress disorder (PTSD), chronic fatigue syndrome, or chronic burnout [Fries et al., 2005; Heim et al., 2000], whereas an elevated HPA axis activity, i.e., an excessive cortisol response to the above-mentioned stimulations, is associated with recurrent depression or Alzheimer's disease [Aihara et al., 2007; Hattinger et al., 1995]. Furthermore, recent human studies using the dexamethasone/corticotropin-releasing hormone (DEX/CRH) test [Heuser et al., 1994], which is considered as a highly sensitive probe of HPA axis activity, have also demonstrated that in adults without current neuropsychiatric disorders, a history of adverse parenting experiences during childhood and adolescence, such as physical, sexual, or emotional abuse, are significantly associated with an attenuated or an excessive cortisol response to DEX/CRH, suggesting that adverse parenting experiences during neurodevelopmental periods could leave long-lasting endocrinological scars expressed as HPA axis abnormalities in adulthood [Carpenter et al., 2009; Heim et al., 2008].

Recent human neuroimaging studies have suggested that adults with childhood maltreatment-related PTSD show a significant gray matter (GM) volume reduction in the hippocampus compared with controls [Bremner et al., 2003; Stein et al., 1997; Woon and Hedges, 2008]. The hippocampus, which is located in the medial temporal lobe and involved in learning and memory function, is believed to be modulated by the HPA axis through the effects of its two corticoid receptors (i.e., the mineralocorticoid receptor and the glucocorticoid receptor) and to provide negative feedback to the HPA axis [Conrad, 2008]. The neurotoxic effects of chronic HPA axis hyperactivity on the hippocampal structure have been well documented, e.g., cortisol hypersecretion in response to the DEX or DEX/CRH test correlates with hippocampal GM volume reduction in patients with depression and dementia [O'Brien et al.,

1996], and hippocampal neuronal atrophy is found after high-dose cortisol administration in animals [Uno et al., 1994]. Therefore, it has been assumed in some studies that adverse parenting experiences during childhood and adolescence could induce chronic HPA axis hyperactivity, resulting in neurodevelopmental abnormalities, such as a hippocampal GM volume reduction in adulthood [Bremner et al., 2003; Stein et al., 1997]. Similarly, chronic HPA axis hypoactivity is suggested to induce a reduction of hippocampal GM volume in rats [Schubert et al., 2008]. Considering this animal study together with the above-mentioned previous human research showing a significant association between a history of adverse parenting experiences and adulthood HPA axis hypoactivity, it might be hypothesized that adverse parenting experiences during childhood and adolescence could induce chronic HPA axis hypoactivity, resulting in an increased risk of hippocampal GM volume reduction with time. However, the associations among adverse parenting experiences during childhood and adolescence, HPA axis hypoactivity, and brain development, particularly hippocampal development, have been insufficiently investigated in humans.

Recently, Engert et al. [2010a,b] have carried out two notable studies regarding the associations among parenting experiences during childhood and adolescence, HPA axis activity, and hippocampal GM volume using the parental bonding instrument (PBI; a retrospective self-report scale to rate the attitudes of parents during the first 16 years) and cortisol response to psychosocial stress tasks. One study revealed that in young adults (aged, 18–30) without psychiatric disorders, those with a maternal medium-care score showed a significantly excessive cortisol response to a psychosocial stress task (i.e., the Trier Social Stress Test; TSST) [Kirschbaum et al., 1993] compared with those with maternal low- and high-care scores, suggesting that maternal low- and high-care scores, but not medium-care score during childhood and adolescence, are associated with HPA axis hypoactivity [Engert et al., 2010b]. Another study based on cortisol response to a psychosocial stress task (i.e., The Montreal Imaging Stress Task; MIST) [Dedovic et al., 2005] suggests a neurodevelopmental model, i.e., lower parental care score during childhood and adolescence induces an increasing adulthood HPA axis activity via hippocampal GM volume reduction in elderly adults (aged, 60–80) without psychiatric disorders

[Engert et al., 2010a]. These two significant studies provide beneficial evidence for associations among parenting experiences during childhood and adolescence, HPA axis activity, and hippocampal GM volume. However, these studies may have included subjects with HPA axis hyper-, normo- to hypoactivities as study samples because of the absence of an assessment of the subjects using neuroendocrine challenge tests, such as the DEX or DEX/CRH test. Considering the above-mentioned animal studies suggesting both HPA axis hypo- and hyperactivities are associated with hippocampal GM volume reduction or childhood parenting experience [Heim et al., 2001, 2008; Schubert et al., 2008; Uno et al., 1994], our understanding regarding associations of HPA axis hypoactivity with parenting experiences during childhood and adolescence and hippocampal GM development could be further improved by selecting individuals without HPA axis hyperactivity using neuroendocrine challenge tests and examining such individuals as study samples.

In this preliminary study, we used a cross-sectional design. Our subjects were healthy young adults with the following feature: no cortisol hypersecretion in response to the DEX (0.5 mg) test, no history of traumatic events, or no past or current conditions of significant medical illness or neuropsychiatric disorders. We aimed to examine the associations among PBI scores [Parker and Hadzi-Pavlovic, 1992; Parker et al., 1979], stimulated cortisol concentration in response to the DEX (0.5 mg)/CRH test, and GM volume focusing on the hippocampus by magnetic resonance imaging (MRI) with voxel-based morphometry (VBM) [Ashburner and Friston, 2000].

MATERIALS AND METHODS

Subjects

Forty-eight normal young adults, aged 18–35 years, were recruited from Gunma Prefecture, Japan, in accordance with the following exclusion criteria: history of a significant medical illness (e.g., neurological or endocrine diseases), a personality disorder, or a psychiatric illness (e.g., PTSD, schizophrenia, anxiety disorders, adjustment disorders, or mood disorders), history of a traumatic event (e.g., serious accident, physical, or sexual abuse), chronic alcoholism or substance abuse, or current chronic medication, in addition to parental divorce or history of a psychiatric illness within a participant's first-degree relatives. Furthermore, subjects were excluded from this study if they worked night shift. To exclude the subjects with past or current major mental disorders or personality disorders, the Structured Clinical Interview for DSM-IV Axis I Disorders [First et al., 1996] and that for Axis II Disorders [First et al., 1997] were used. Then, we excluded subjects with a possible HPA axis hyperactivity on the basis of their plasma cortisol concentration at first blood sampling (1400 h; basal cortisol concentration) in the DEX/CRH test, the details of which are shown in DEX/CRH Test Procedure.

By this exclusion process, based on basal cortisol concentration, 8 of the 48 initial subjects were excluded from this study.

Finally, 40 subjects, aged 18–35 years (mean, 27.2 ± 5.2 ; 20 females), were enrolled in this study. All the 40 subjects were free of any medications for at least one month. Two subjects who smoked cigarettes were included in the study. In all the subjects, parental household income per year during a participant's childhood and adolescence was more than 5,000,000 yen ($\sim$ \$ 50,000), which is above the mean household income in Japan, as reported by the Japan Ministry of Health (<http://www.mhlw.go.jp/toukei/itiran/dl/g02.pdf>).

All the subjects enrolled in this study were right-handed as assessed using the Edinburgh Handedness Inventory [Oldfield, 1971]. All the subjects provided their written informed consent. The study protocol was approved by the Ethics Committee of Gunma University. Supporting Information Tables Ia,b and IIa,b show the demographic characteristics of parental low- and high-PBI-score groups, or parental low-, medium-, and high-PBI-score groups in this study subjects.

Experimental Procedure

On the first day of this examination and the following day, 48 subjects underwent the DEX/CRH test. Within one month after the DEX/CRH test, 40 subjects without cortisol hypersecretion in response to DEX (0.5 mg) (see DEX/CRH Test Procedure) filled out self-report questionnaires for psychological measurements including PBI and underwent MRI. On the same day, considering that the hippocampus is closely associated with cognitive functions [Conrad, 2008], the memory and attention function of the subjects were assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB) with a computer [Morris et al., 1987].

Psychological Measurements

PBI

The perceived parental rearing styles were assessed using the Japanese version of PBI. PBI is a self-report scale with 25 items to rate paternal or maternal attitude during the first 16 years, and has four items comprising care (range, 0–36 points) and overprotection (range, 0–39 points) factors, i.e., paternal care, maternal care, paternal overprotection, and maternal overprotection, the cut-off points of which are <24, <27, <12.5, and <13.5, respectively [Parker and Hadzi-Pavlovic, 1992; Parker et al., 1979]. The care factor has one pole defined by care and affection in the parent-child relationship and the other defined by indifference and rejection. The overprotection factor has one pole defined by control, overprotection, and intrusion and the other defined by encouragement of independence and autonomy. Furthermore, the overprotection

factor has been reported to be further divided into two different subscales, i.e., score for denial of psychological autonomy (range, 0–21 points) and that for encouragement of behavioral freedom (range, 0–18 points) [Murphy et al., 1997].

Harvard trauma questionnaire (HTQ)

Furthermore, HTQ was used for the screening for PTSD, which is a reliable, validated, culturally sensitive instrument for measuring trauma and PTSD [Mollica et al., 1996]. The standard cut-off item score of 2.0 or higher was used to indicate a probable PTSD diagnosis [Mollica et al., 2001].

Zung self-rating depression scale (SDS)

For evaluation of depressive symptoms, all the subjects completed a questionnaire corresponding to the depression scale taken from SDS, which is a widely used inventory that consists of 20 items on a four-point scale, with a lower score representing a more favorable psychological state [Zung, 1965].

Japanese version of national adult reading test

The Japanese version of the National Adult Reading Test (JNART) was then used to estimate premorbid IQs [Matsuoka et al., 2006; Nelson, 1982].

Parental socioeconomic status (SES)

The subjects' SES and parental SES were assessed using the Hollingshead scale [Hollingshead, 1965].

Assessment of Cognitive Functional

CANTAB, which consists of neuropsychological tests using a touch screen portable computer, was administered to all the subjects for the assessment of cognitive function (the Cambridge Cognition website: <http://www.cantab.com/camcog/default.asp>) [Morris et al., 1987]. Three tests of CANTAB were used in this study as follows: (1) Pattern recognition memory (PRM) is a test of visual recognition memory in a two-choice forced discrimination paradigm. The subject is presented with a series of 12 visual patterns, one at a time, at the centre of the screen. These patterns are designed such that they cannot easily be given verbal labels. In the recognition phase, the subject is required to choose between a pattern he/she has already seen and a novel pattern. In this phase, the test patterns are presented in the reverse order to the original order of presentation. (2) Spatial recognition memory (SRM) tests visual spatial memory in a two-choice forced discrimination paradigm. The subject is presented with a white square, which appears in sequence at five different locations on the screen. In the recognition phase, the subject sees a series of

five pairs of squares, one of which is in a place previously seen in the presentation phase. The other square is in a location not seen in the presentation phase. (3) Rapid visual information processing (RVIP) is a visual continuous performance task, using digits instead of letters. A white box appears in the centre of the computer screen, inside which digits, from 2 to 9, appear in a pseudorandom order, at a rate of 100 digits per minute. Subjects are instructed to detect target sequences of digits (for example, 2-4-6, 3-5-7, 4-6-8) and to register responses using the press pad.

DEX/CRH Test Procedure

We modified the original method of Holsboer et al. [1995] as follows. A low dose of DEX (0.5 mg; Japanese brand name, "Dekadoron"; produced by Merck & Co., Inc., NJ) was administered orally at 2300 h, and at approximately 1330 h the following day each patient was instructed to lie in a supine position and a heparinized catheter was inserted into a cubital vein. At 1400 h, the first blood sample was drawn through the intravenous catheter, after which 100 µg of human CRH (Japanese brand name, "Mitsubishi"; produced by Mitsubishi Tanabe Pharma Corporation., Inc., Osaka, Japan) was administered intravenously instead of by CRH injection at 1500 h, as in previous studies [Heuser et al., 1994; Holsboer et al. 1995]. Blood samples were drawn again through the intravenous catheter at 1330 h, 1345 h, 1500 h, and 1515 h. Blood samples were immediately centrifuged and stored at -80°C . Plasma cortisol and adrenocorticotrophic hormone (ACTH) concentrations were measured by radioimmunoassay at Mitsubishi Chemical Medience Corporation, Tokyo, Japan.

The results were evaluated in accordance with the method of Holsboer et al. [1995]. "Basal cortisol" and "basal ACTH" concentrations were defined as the plasma cortisol and ACTH concentrations at 1400 h of blood collection. After the area under the time course curve (AUC) was calculated by trapezoidal integration. "AUC_{net} cortisol" or "AUC_{net} ACTH" (corrected baseline of plasma cortisol or ACTH concentration, respectively) was computed as a measure of cortisol response to CRH injection. Thus, basal cortisol concentration (or basal ACTH) reflects the suppressive effect of DEX (0.5 mg) administered the day before, whereas AUC_{net} cortisol (or AUC_{net} ACTH) reflects the additional effects of CRH injection.

In this study, subjects with basal cortisol concentration higher than 4 µg/100 ml were excluded in accordance with the cut-off value that indicates nonsuppression of response to DEX (0.5 mg), which is considered to indicate HPA axis hyperactivity in the Japanese population [Matsunaga and Sarai, 2000].

MRI Acquisition

Brain MRI was performed using a Siemens 1.5-T Magnetom Symphony (Siemens, Erlangen, Germany). A three-dimensional gradient-echo sequence (fast low-angle shot, FLASH) yielding 160–192 contiguous slices of 1.0 mm

thickness in the axial plane was used for volume analysis. This sequence provided high-resolution T1-weighted images with good contrast between GM and the white matter (WM). Imaging parameters were as follows: echo time = 5 msec; repetition time = 24 msec; flip angle = 40°; field of view = 256 mm; matrix size = 256 × 256; voxel size = 1 × 1 × 1 mm³.

Image Analysis and Statistical Analyses

For voxel-based morphometry (VBM) [Ashburner and Friston, 2000], T1-weighted volumetric images were analyzed using SPM5 (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 2008a (MathWorks, Natick, MA, USA) to carry out VBM5.1 (<http://dbm.neuro.uni-jena.de/vbm/download>) using default parameters (DCT cutoff = 25 mm; nonlinear regularization = 1, 16 iterations). Each image was inspected for reconstruction artifacts. The VBM5.1 method combines spatial normalization, segmentation, and volumetric modulation. Each image was segmented into GM, WM, and cerebrospinal fluid using the Hidden Markov Random Field (HMRF) model, normalized to the International Consortium for Brain Mapping (ICBM) 152 template (Montreal Neurological Institute; MNI) (nonlinear transformation), and modulated with preservation of the total amount of GM. GM image segments were inspected for segmentation artifacts, then smoothed using an isotropic Gaussian kernel of 12 mm full width at half maximum (FWHM) [Mechelli et al., 2005]. An absolute threshold mask of 0.10 was used to avoid possible edge effects around the border between GM and WM. The significance level was set at $P < 0.05$ corrected for multiple comparisons at the cluster level and a familywise error rate of $P < 0.05$ at the voxel level (FWER-corrected $P < 0.05$) with adjustments for age, gender, and total GM volume.

Moreover, we performed region-of-interest (ROI) analysis by small volume correction to investigate the correlation between PBI scores or results of the DEX/CRH test and regional GM volume of the bilateral hippocampi by automated anatomical labeling (AAL) of the Wake Forest University (WFU) Pickatlas [Maldjian et al., 2003, 2004; Tzourio-Mazoyer et al., 2002], which provided an atlas-based method of generating ROIs (Fig. 2A). Furthermore, to determine total hippocampal GM volume in each subject, the mean signal intensities on T1-weighted images were extracted from the bilateral hippocampi for each subject using the MarsBar toolbox (<http://marsbar.sourceforge.net>) [Brett et al., 2002]. Total hippocampal GM volume, including the entire GM volume in the bilateral hippocampi was determined from the mean signal intensities on T1-weighted images within the bilateral hippocampi.

Statistical Analysis

Spearman's rho correlation and partial rank correlation with adjustments for confounding factors (i.e., age and

gender, or age, gender, and total GM volume) were carried out to assess relationships among demographic characteristics, psychological and cognitive parameters, results of the DEX/CRH test, and total hippocampal GM volume. Student's *t*-test was used to assess gender differences. One-way analysis of variance (ANOVA) and analysis of covariance with adjustments for confounding factors were used to compare AUC_{net} cortisol or total hippocampal GM volume between low- and high-PBI score classified on the basis of the cut-off score of each PBI item (i.e., paternal care, paternal overprotection, maternal care or maternal overprotection) [Parker and Hadzi-Pavlovic, 1992; Parker et al., 1979], using SPSS for Windows ver. 12 (SPSS Japan Inc., Tokyo, Japan). Finally, considering a previous PBI study by Engert et al. [2010b] revealing that maternal medium-care score group shows a significantly excessive cortisol response to a psychosocial stress task compared with the maternal low- and high-care score groups, we divided our subjects into three groups (low-, medium-, and high-PBI score groups) on the basis of the ascending order of score of each PBI item, and compared the three groups, by one-way ANOVA with Bonferroni.

RESULTS

Table I shows the demographic characteristics, psychological measures, ACTH and cortisol levels, and hippocampal volumes of the study subjects. No significant gender differences were observed in basal cortisol concentration, AUC_{net} cortisol, and total hippocampal volume. The HTQ scores of all the subjects were below 2.0, which is the standard cut-off score indicating a probable PTSD diagnosis [Mollica et al., 2001].

Spearman's rho correlation showed that neither basal cortisol concentration nor AUC_{net} cortisol correlated with age, education years, SES score, parental SES score, JNART score, depressive symptom score, or scores of cognitive function tests (i.e., PRM, SRM, and RVIP). Moreover, neither basal ACTH concentration nor AUC_{net} ACTH correlated with age, education years, SES score, parental SES score, JNART score, depressive symptom score, or scores of cognitive function tests. AUC_{net} ACTH significantly correlated with AUC_{net} cortisol ($r = 0.634$, $P < 0.01$), and this significance remained after adjustments for age and gender ($r = 0.658$, $P < 0.01$). All PBI scores (i.e., parental care and overprotection scores) and all parental overprotection subscale scores (i.e., score for parental denial of psychological autonomy and paternal encouragement of behavioral freedom) did not correlate with basal ACTH concentration, AUC_{net} ACTH, or basal cortisol concentration. On the other hand, paternal and maternal overprotection scores significantly negatively correlated with AUC_{net} cortisol ($r = -0.356$, $P < 0.05$ and $r = -0.366$, $P < 0.05$, respectively). The significance of these correlations remained after adjustments for age and gender ($r = -0.339$, $P < 0.05$ and $r = -0.353$, $P < 0.05$, respectively). Neither paternal nor maternal care scores significantly correlated with AUC_{net}

TABLE I. Demographic characteristics, psychological measures, ACTH and cortisol levels, and hippocampal volumes of demographic characteristics of study subjects

	Male	Female
Number	20	20
Age (y)	28.4 ± 4.8	25.9 ± 5.3
Education (y)	15.9 ± 1.5	14.6 ± 1.9
SES score (point)	3.4 ± 0.9	3.6 ± 0.7
Parental SES score (point)	2.8 ± 1.6	3.0 ± 1.7
JART—predicted full-scale IQ	103.0 ± 8.3	107.0 ± 9.5
CANTAB (Z-score)		
Pattern recognition memory*	0.01 ± 0.59	0.36 ± 0.40
Spatial recognition memory	0.54 ± 0.65	0.42 ± 1.23
Rapid visual information processing	0.87 ± 0.77	0.82 ± 0.94
Depressive symptom score (point)	35.1 ± 7.8	37.1 ± 8.9
Parental bonding instrument (point)		
Paternal care	23.2 ± 6.8	24.2 ± 4.6
Paternal overprotection	10.6 ± 6.9	10.6 ± 4.9
Maternal care	26.4 ± 5.1	26.6 ± 5.4
Maternal overprotection	10.3 ± 5.9	10.2 ± 6.7
Dexamethasone (0.5mg)/CRH test		
Basal ACTH (ng/ml)	5.6 ± 3.9	4.6 ± 2.2
AUC _{net} ACTH (ng/ml min)	1029.1 ± 483.1	953.9 ± 523.2
Basal cortisol (µg/100ml)	1.6 ± 1.1	1.6 ± 1.2
AUC _{net} cortisol (µg/100ml min)	304.8 ± 179.4	338.8 ± 218.3
Brain tissue to intracranial volume ratio		
Gray matter/intracranial volume	0.457 ± 0.030	0.463 ± 0.025
White matter/intracranial volume	0.315 ± 0.024	0.305 ± 0.028
Cerebrospinal fluid/intracranial volume	0.228 ± 0.031	0.231 ± 0.042
Average gray matter density in bilateral hippocampi	0.487 ± 0.018	0.501 ± 0.021

Abbreviations: SES, socioeconomic status; JART, the Japanese version of the National Adult Reading Test; AUC_{net} cortisol, corrected baseline of plasma cortisol concentration, after the area under the time course curve (AUC) was calculated by trapezoidal integration; CANTAB, the Cambridge Neuropsychological Test Automated Battery, which is expressed as Z-score calculated from the internal normative database of CANTAB, involving 3,000 healthy volunteers. Mean ± SD.

* $P < 0.05$ (Student's *t*-test).

cortisol (Fig. 1). Moreover, all parental overprotection subscale scores did not significantly correlate with AUC_{net} cortisol (Supporting Information Fig. 1). Total hippocampal GM volume significantly positively correlated with AUC_{net} cortisol ($r = 0.402$, $P < 0.05$), and the significance of this correlation remained after adjustments for age, gender, and total GM volume ($r = 0.339$, $P < 0.05$) (Fig. 2C), whereas no significant correlation was observed between total hippocampal GM volume and basal ACTH concentration, AUC_{net} ACTH, or basal cortisol concentration. Total hippocampal GM volume did not significantly correlate with the subjects' and parental SES scores, education years, JNART scores, depressive symptom score, and all PBI scores and parental overprotection subscale scores. Total hippocampal GM volume significantly negatively correlated with maternal overprotection scores ($r = -0.400$, $P < 0.05$) and significantly positively correlated with maternal care score ($r = 0.334$, $P < 0.05$, respectively), but these significances did not remain after adjustments for age, gender, and total GM volume ($r = -0.194$, NS and $r = 0.306$, NS, respectively). Furthermore, total hippocampal GM volume significantly negatively correlated with

the number of incorrect answers in PRM of cognitive function tests ($r = -0.340$, $P < 0.05$), but this significance did not remain after adjustments for age, gender, and total GM volume ($r = -0.165$, NS). Total hippocampal GM volume did not significantly correlate with SRM and RVIP scores in cognitive function tests.

Correlation of PBI Scores (i.e., Care and Overprotection Scores) or AUC_{net} Cortisol With Regional GM Volume in Whole Brain Determined by VBM

When we estimated the regional GM volume of areas within the entire brain that statistically significantly correlated with each PBI score (i.e., paternal care, paternal overprotection, maternal care, or maternal overprotection score), no significant positive or negative correlation was found in any GM area (FWER-corrected $P > 0.05$). Similarly, when we estimated the regional GM volume of areas within the entire brain that were statistically significantly associated with AUC_{net} cortisol, no significant positive or

Figure 1.

Associations between scores of PBI (i.e., care and overprotection) and AUC_{net} cortisol, or AUC_{net} ACTH. Blue circles indicate male subjects and green circles indicate female subjects. Spearman's rho correlation, $P < 0.05$.

negative correlation was found in any GM area (FWER-corrected $P > 0.05$).

Small-Volume Correction to Estimate Correlation of PBI Scores (i.e., Care and Overprotection Scores) or AUC_{net} Cortisol With Regional GM Volume Within Bilateral Hippocampi Determined by VBM With AAL of WFU Pickatlas

PBI scores and regional GM volume focusing on bilateral hippocampi

When we estimated the regional GM volume of areas focusing on the bilateral hippocampi that were statistically significantly associated with each PBI score, no significant positive or negative correlation was found in any GM area within the bilateral hippocampi (FWER-corrected $P > 0.05$).

AUC_{net} cortisol and regional GM volume focusing on bilateral hippocampi

An ROI analysis focusing on the bilateral hippocampi showed that AUC_{net} cortisol positively correlated with GM

volume in the left hippocampus (FWER-corrected $P < 0.05$), after adjustments for age, gender, and total GM volume (Fig. 2B, Table II). On the other hand, no significant negative correlation between AUC_{net} cortisol and GM area within the bilateral hippocampi was found (FWER-corrected $P > 0.05$).

Estimation of Differences in AUC_{net} Cortisol and Total Hippocampal GM Volume between Parental Low- and High-PBI-Score Groups (i.e., Care and Overprotection Scores)

AUC_{net} cortisol

One-way ANOVA showed no significant association in AUC_{net} cortisol between the paternal low-care-score group ($n = 17$) and the paternal high-care-score group ($n = 23$) ($F = 0.323$, $P = 0.573$). Moreover, no significant association in AUC_{net} cortisol was observed between the maternal low-care-score group ($n = 19$) and the maternal high-care-score group ($n = 21$) ($F = 0.560$, $P = 0.459$). The paternal low-overprotection-score group ($n = 24$) has a significantly higher AUC_{net} cortisol than the paternal high-overprotection-score group ($n = 16$) ($F = 7.108$, $P = 0.011$), and this significance remained after adjustments for age and

Figure 2.

Panel (A) shows the brain region including the bilateral hippocampi (blue area), masked by automated anatomical labeling (AAL) of the Wake Forest University (WFU) Pickatlas, which provided an atlas-based method of generating ROIs. Panel (B) shows the brain region that significantly positively correlated with AUC_{net} cortisol, as determined by ROI analysis focusing on the hippocampus. The blue lines indicate the left tail in the hippocampus. The statistical threshold was set at $P < 0.05$ corrected for multiple comparisons at the cluster level and a

familywise error rate of $P < 0.05$ at the voxel level (FWER-corrected $P < 0.05$) with adjustments for age, gender, and total GM volume. Panel (C) shows the association between AUC_{net} cortisol and total hippocampal GM volume, as determined by the mean signal intensities on T1-weighted images within the bilateral hippocampi. Blue circles indicate male subjects and green circles indicate female subjects. Spearman's rho correlation, $P < 0.05$.

TABLE II. Brain region showing significant positive correlation between AUC_{net} cortisol and average gray matter density in bilateral hippocampi after adjustments for age, gender, and total gray matter volume

	Cluster level		Peak coordinates ^a			Voxel level	
	Corrected <i>P</i>	<i>k</i>	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i>	FWER-corrected <i>p</i>
L hippocampus	0.025	137	−25	−38	−4	4.31	0.018

^a*x*, *y*, *z*, are the stereotaxic coordinates, as given in Montreal Neurological Institute Atlas.

L, left. The significance level was set at $P < 0.05$ corrected for multiple comparisons at the cluster level, and $P < 0.05$ familywise error rate (FWER-corrected) at the voxel level.

gender ($F = 5.794$, $P = 0.021$). Also, the maternal low-overprotection-score group ($n = 28$) has a significantly higher AUC_{net} cortisol than the maternal high-overprotection-score group ($n = 12$) ($F = 4.384$, $P = 0.043$), and this significance remained after adjustments for age and gender ($F = 4.277$, $P = 0.046$).

Total hippocampal GM volume

One-way ANOVA showed no significant association in total hippocampal GM volume between the paternal low-care-score and paternal high-care-score groups ($F = 0.035$, $P = 0.852$). Also, no significant association in total hippocampal GM volume was observed between the maternal low-care-score and maternal high-care-score groups ($F = 3.858$, $P = 0.057$). The paternal low-overprotection-score group showed a significantly greater total hippocampal GM volume than the paternal high-overprotection-score group ($F = 5.494$, $P = 0.024$), but this significance did not remain after adjustments for age and gender ($F = 3.194$, $P = 0.083$). Also, the maternal low-overprotection-score group has a significantly greater total hippocampal GM volume than maternal high-overprotection-score group ($F = 7.694$, $P = 0.009$), but this significance did not remain after adjustments for age and gender ($F = 2.834$, $P = 0.101$) (see Supporting Information Table 1a,b).

Estimation of Differences in AUC_{net} Cortisol or Total Hippocampal GM Volume Among Parental Low-, Medium-, and High-PBI-Score Groups (i.e., Care and Overprotection Scores)

One-way ANOVA with Bonferroni showed no significant associations in AUC_{net} cortisol among low-, medium-, and high-score groups for each PBI items, i.e., paternal care (low: $n = 13$; range, 1–22 points; medium: $n = 16$; range, 23–26 points; and high: $n = 11$; range, 27–33 points), paternal overprotection (low: $n = 13$; range, 0–8 points; medium: $n = 12$; range, 9–13 points; and high: $n = 15$; range, 14–25 points), maternal care (low: $n = 14$; range, 15–25 points; medium: $n = 13$; range, 26–29 points; and high: $n = 13$; range, 30–33 points), or maternal overprotection (low: $n = 13$; range, 1–6 points; medium: $n = 15$; range, 9–13 points; and high: $n = 12$; range, 14–23 points). Also, no significant associations in total hippocampal vol-

ume were observed among the low-, medium- and high-score-groups for each PBI item (Supporting Information Table 2a,b).

DISCUSSION

From the results of the correlation analyses and comparisons between the low- and high-PBI-score groups in this study, a significant negative association was found between parental overprotection scores and AUC_{net} cortisol, suggesting that parental overprotection during the first 16 years increases the risk of dampening of cortisol reactivity, i.e., HPA axis hypoactivity, in adulthood. On the other hand, parental care scores did not correlate with AUC_{net} cortisol. A significant positive correlation between cortisol response to DEX/CRH and GM volume in the hippocampus was observed (Fig. 2B,C), suggesting that HPA axis hypoactivity is associated with the hippocampal GM volume reduction, which is consistent with previous animal studies that demonstrated that chronic HPA axis hypoactivity after adrenalectomy contributes to the GM volume reduction of the hippocampus [Fries et al., 2005; Schubert et al., 2008]. However, parental care and overprotection scores were not significantly associated with total or regional hippocampal GM volume. In addition, no significant difference was observed in AUC_{net} cortisol or total hippocampal GM volume between parental low-, medium-, and high-PBI-score groups.

As far as we know, there has been no study describing an association between PBI scores and cortisol response to DEX/CRH test. In previous research on cortisol response to neuroendocrine challenge, association between HPA axis activity and parenting experiences during childhood and adolescence was studied previously in nondepressed human adults with a history of early-life stress, using original interview, structured interview (i.e., the Early Trauma Inventory), or childhood trauma questionnaire (CTQ; a self-report scale containing items of emotional, physical, and sexual abuses)] to measure parenting experiences during childhood and adolescence [Carpenter et al., 2009; Heim et al., 2001, 2008], but the results of such studies are inconsistent. Heim et al. [2001] described that childhood physical or sexual abuse is associated with lower basal cortisol and stimulated plasma cortisol concentrations in response to iv CRH and ACTH challenge tests. Another

study has demonstrated that childhood sexual or physical abuse is associated with an increased plasma ACTH concentration and enhanced cortisol responses to DEX/CRH [Heim et al., 2008]. Carpenter et al. [2009] revealed using CTQ that childhood emotional abuse is associated with the dampening of cortisol response to DEX/CRH. On the basis of this finding together with the above-mentioned previous findings, they proposed that different types of parenting experience during childhood and adolescence (emotional, physical, or sexual abuse) might portend different consequences with regard to HPA axis activity in adulthood, that is, childhood emotional or physical abuse is likely associated with HPA axis hypoactivity in adulthood, whereas childhood sexual abuse is likely associated with HPA axis hyperactivity in adulthood. CTQ is one of the widely used self-report measurements to examine the association of neuroendocrine challenge test with parenting experiences during childhood and adolescence. Although it is expected that both CTQ and PBI should have been scored and analyzed in this study, we were unable to administer CTQ unfortunately, because the Japanese version of which has not yet been developed. Different from studies using neuroendocrine challenge tests, Engert et al. [2010b] described that in young adults without psychiatric disorders, the maternal medium-care-score group shows a greater cortisol response to a psychosocial task [i.e., TSST; a 10-min free speech in front of panelists and a camera] [Kirschbaum et al., 1993], compared with the maternal low- and high-care-score groups. However, we failed to find significant associations in all comparisons of AUC_{net} cortisol among the parental low-, medium- and high-care-score groups in this study. As the reasons for this discrepancy between the findings of the two studies, the following are proposed. Because in this study we focused on the associations of HPA axis hypoactivity with PBI score and hippocampal GM volume, we excluded 8 of the 48 subjects who possibly showed HPA axis hyperactivity, as determined by the DEX test, which measures the negative feedback effects of DEX via anterior pituitary glucocorticoid receptor activation [Pariante and Miller, 2001]. On the other hand, the study by Engert et al. [2010b] may have included subjects with HPA axis hyperactivity as study samples, because their subjects were not subjected neuroendocrine challenge tests such as the DEX test; thus, data of those subjects might have affected their findings. Second, the DEX/CRH test contains the suppressive effect of DEX on the pituitary-adrenal response to CRH and the facilitative effect of CRH on ACTH and cortisol release [Kunugi et al., 2006], whereas a cortisol response to TSST could reflect an ability to cope with psychosocial stress in each individual, because TSST combines uncontrollable and socioevaluative elements [Dickerson and Kemeny, 2004]. On the basis of above-mentioned characteristic features of TSST, Engert et al. [2010b] described that in their study, the medium-care-score group likely has the adaptive coping ability and an average cortisol response to psychosocial stress, but the low- and high-care-score groups

may tend to show HPA axis hypoactivity, resulting in the maternal medium-care-score group, showing a significantly excessive cortisol response to TSST than the maternal low- and high-care-score groups. Finally, as a major cause, the effect of statistical type II error on our results in this study must be considered. In this study, rank correlation analyses and comparison between two groups (i.e., low- and high-PBI-score groups) showed significant associations between AUC_{net} cortisol and parental overprotection scores of PBI, but no significant association was found in all comparisons of AUC_{net} cortisol among low-, medium- and high-PBI-score groups, suggesting that type II error could affect the results of comparison among the three groups, owing to the small number of our subjects. Thus, further analysis with a larger number of subjects is required.

As a possible explanation of how chronic stress during childhood and adolescence induces adulthood cortisol hyporeactivity in response to DEX/CRH, it is hypothesized on the basis of a developmental model that a trajectory of initial hyperactivation of the HPA system could progress to a state of chronic adrenal stress hypoactivity [Fries et al., 2005; Pryce et al., 2005] as a type of counter-regulatory adaptation after acute or sustained exposure to excessive ACTH levels during a stressful early developmental period [Miller et al., 2007]. As several previous animal studies reported CRH hypersecretion from the hypothalamus and adaptive down-regulation of CRH receptors under continuous stress [Hauger et al., 1988; Makino et al., 1994], CRH receptor downregulation might be involved in reduced ACTH levels and an attenuated cortisol response to CRH. In addition, reduced biosynthesis or depletion at several levels of the HPA axis (CRH, ACTH, and/or cortisol) after enhanced secretions of these hormones under continuous stress might contribute to cortisol hyporeactivity in response to DEX/CRH [Heim et al., 2000]. Furthermore, an increased sensitivity to the HPA axis for a negative feedback is suggested to attenuate the cortisol response [Yehuda et al., 1991].

A significant positive association was found between HPA axis hypoactivity and hippocampal GM volume reduction in healthy young adults in this study. The mechanisms by which chronic HPA axis hypoactivity contributes to the GM volume reduction of the hippocampus are explained as follows. Because *myo*-inositol level is considered to be an astrocyte marker and *myo*-inositol induces cell membrane metabolism and osmoregulation, removal of cortisol is reported to induce the decrease in *myo*-inositol level in the hippocampus [Brand et al., 1993; Heilig et al., 1989; Schubert et al. 2008]. Thus, it is suggested that HPA axis hypoactivity induces an altered astrocyte metabolism or an electrolyte disturbance such as hyponatremia, leading to hippocampal neurodegeneration and insufficient GM development in the hippocampus with time [Fries et al., 2005].

The results of our study seem to support the hypothesis that parenting overprotection during childhood and

adolescence could induce the reduction of hippocampal GM volume in adulthood via HPA axis hypoactivity. However, we failed to show the direct association of PBI scores with total or regional hippocampal GM volume. Some previous research showed a significant association between childhood maltreatment and reduction of hippocampal GM volume in adulthood [Bremner et al., 2003; Stein et al., 1997; Woon and Hedges, 2008]. These studies have included patients with current PTSD related to maltreatment as study subjects, which differed from our study. Thus, the above-mentioned statistical insignificance in this study might result from our sampling method, i.e., only adults without a history of childhood traumatic events and current psychiatric disorders including PTSD were enrolled. As another possibility, statistical type II error might be the cause of this absence of significant association, owing to the small sample size of this study. Indeed, Pearson's simple correlation showed a significant negative relationship between parental overprotection score and total hippocampal GM volume in this study, but this significance did not remain after adjustments for age, gender, and total GM volume.

Another study by Engert et al. [2010a] revealed in the elderly adults without psychiatric disorders a significant positive association between hippocampal GM volume and parental care score of PBI, as well as a significant negative association between hippocampal GM volume and cortisol response to psychosocial stress task (MIST; subjects perform an arithmetic task and receive a negative feedback to its performance) [Dedovic et al., 2005]. On the basis of these findings, they suggested a notable neurodevelopmental model, i.e., parental low-care during childhood and adolescence induces an adulthood HPA axis hyperactivity via hippocampal GM volume reduction. On the other hand, our finding in this study showed no significant association between PBI scores and hippocampal GM volume reduction. Different from our subjects in this research, the study by Engert et al. [2010a,b] did not exclude subjects with a possible HPA axis hyperactivity. As a result, the associations of HPA axis hyperactivity with hippocampal GM volume and PBI score might mask the statistically significant associations of HPA axis hypoactivity with them. Furthermore, the age difference between the subjects in the study by Engert et al. [2010a] and those in our study (i.e., elderly or young adults) should be considered because a number of studies of humans and experimental animals provide evidence that HPA axis hyperactivity contributes to degeneration of neurons, including those in the hippocampus, associated with aging [Ferrari et al., 1995; Lupien et al., 1998; Sapolsky, 1999; Swaab et al., 2005].

In this study, we used 0.5 mg of DEX in the DEX/CRH test because a higher sensitivity of the 0.5 mg DEX test has been reported in the Japanese/Asian populations [Matsunaga and Sarai, 2000]. However, there is no evidence that the DEX (0.5 mg)/CRH test is recommended for any populations on the basis of their DEX/CRH sensitivity. In

most of the previous studies using the DEX/CRH test, researchers used 1–1.5 mg of DEX to determine HPA axis hyperactivity in patients with several psychiatric disorders, such as major depression [Kunugi et al., 2006], whereas the DEX test at a low dose of DEX (0.5 mg) has been frequently performed to estimate HPA axis hypoactivity in patients with PTSD [Yehuda et al., 1993]. Accordingly, further research using the DEX/CRH test at conventional doses of DEX (1–1.5 mg) will be required to examine associations among parenting experiences during childhood and adolescence, HPA axis hypoactivity, and hippocampal GM volume.

In addition to sample size, subject selection, and the dose of DEX in the DEX/CRH test there were some limitations in this study as follows. PBI is an instrument for measuring retrospectively recalled parental behaviors, which leaves it open to possible influences of current mood state or recall bias. Moreover, we were unable to obtain a causal relationship among parenting overprotection during childhood and adolescence, HPA axis hypoactivity, and hippocampal GM volume reduction, because of the cross-sectional study design and the lack of subjects in other age ranges, i.e., childhood and adolescence. In addition, other lines of evidence showed that variation of the corticotropin-releasing hormone receptor (CRHR1) gene could moderate the effect of childhood maltreatment on cortisol response to DEX/CRH in adulthood [Tyrka et al., 2009]. Thus, further research with a larger sample size, including patients with past and current PTSD, using other doses of DEX in the DEX/CRH test, and taking heredity into consideration (i.e., CRHR1 gene types) will be required.

In conclusion, statistically significant associations were found between parental overprotection during childhood and adolescence and adulthood HPA axis hypoactivity, and between HPA axis hypoactivity and hippocampal GM volume reduction in healthy young adults, but no significant relationship was observed between any PBI scores and adulthood hippocampal GM volume. The findings of this study will increase our understanding of associations among parenting experiences during childhood and adolescence, HPA axis activity, and hippocampal GM development.

ACKNOWLEDGMENTS

All the authors declare that they have no actual or potential conflicts of interest. They are deeply grateful to all the subjects as well as all the medical staff members involved in this project. The funding agency played no role in the study design, collection, analysis or interpretation of data, writing of the report, or decision to submit the paper for publication.

REFERENCES

- Aihara M, Ida I, Yuuki N, Oshima A, Kumano H, Takahashi K, Fukuda M, Oriuchi N, Endo K, Matsuda H, Mikuni M (2007):

- HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Res* 155:245–256.
- Ashburner J, Friston K (2000): Voxel-based morphometry—The methods. *Neuroimage* 11:805–821.
- Brand A, Richter-Landsberg C, Leibfritz D (1993): Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci* 15:289–298.
- Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, Khan S, Vaccarino LV, Soufer R, Garg PK, Ng CK, Staib LH, Duncan JS, Charney DS (2003): MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *Am J Psychiatry* 160:924–932.
- Brett M, Anton J, Valabregue R, Poline J (2002): Region of interest analysis using an SPM toolbox [abstract]. Paper presented at the Eighth International Conference on Functional Mapping of the Human Brain, Sendai, Japan.
- Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH (2009): Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biol Psychiatry* 66:69–75.
- Claes SJ (2004): Corticotropin-releasing hormone (CRH) in psychiatry: From stress to psychopathology. *Ann Med* 36:50–61.
- Conrad CD (2008): Chronic stress-induced hippocampal vulnerability: The glucocorticoid vulnerability hypothesis. *Rev Neurosci* 19:395–411.
- Dedovic K, Renwick R, Mahani NK, Engert V, Lupien SJ, Pruessner JC (2005): The Montreal imaging stress task: Using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *J Psychiatry Neurosci* 30:319–325.
- Dickerson SS, Kemeny ME (2004): Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychol Bull* 130:355–391.
- Engert V, Buss C, Khalili-Mahani N, Wadiwalla M, Dedovic K, Pruessner JC (2010a) Investigating the association between early life parental care and stress responsivity in adulthood. *Dev Neuropsychol* 35:570–581.
- Engert V, Efanov SI, Dedovic K, Duchesne A, Dagher A, Pruessner JC (2010b) Perceived early-life maternal care and the cortisol response to repeated psychosocial stress. *J Psychiatry Neurosci* 35:370–377.
- Ferrari E, Magri F, Dori D, Migliorati G, Nescis T, Molla G, Fioravanti M, Solerte SB (1995): Neuroendocrine correlates of the aging brain in humans. *Neuroendocrinology* 61:464–470.
- First MB, Spitzer RL, Gibbon M, Williams JBW (1996): Structured Clinical Interview for DSM-IV Axis I Disorders, Version 2.0. New York: New York State Psychiatric Institute.
- First MB, Spitzer RL, Williams JBW, Gibbon M (1997): Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II) User's Guide and Interview. Washington DC: American Psychiatric Press.
- Fries E, Hesse J, Hellhammer J, Hellhammer DH (2005): A new view on hypocortisolism. *Psychoneuroendocrinology* 30:1010–1016.
- Hatzinger M, Z'Brun A, Hemminger U, Seifritz E, Baumann F, Holsboer-Trachsler E, Heuser IJ (1995): Hypothalamic-pituitary-adrenal system function in patients with Alzheimer's disease. *Neurobiol Aging* 16:205–209.
- Hauger RL, Millan MA, Lorang M, Harwood JP, Aguilera G (1988): Corticotropin-releasing factor receptors and pituitary adrenal responses during immobilization stress. *Endocrinology* 123:396–405.
- Heilig CW, Stromski ME, Blumenfeld JD, Lee JP, Gullans SR (1989): Characterization of the major brain osmolytes that accumulate in salt-loaded rats. *Am J Physiol* 257:F1108–F1116.
- Heim C, Ehler U, Hellhammer DH (2000): The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 25:1–35.
- Heim C, Newport DJ, Bonsall R, Miller AH, Nemeroff CB (2001): Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *Am J Psychiatry* 158:575–581.
- Heim C, Mletzko T, Purselle D, Musselman DL, Nemeroff CB (2008): The dexamethasone/corticotropin-releasing factor test in men with major depression: Role of childhood trauma. *Biol Psychiatry* 63:398–405.
- Heuser I, Yassouridis A, Holsboer F (1994): The combined dexamethasone/CRH test: A refined laboratory test for psychiatric disorders. *J Psychiatr Res* 28:341–356.
- Hollingshead AB (1965): Two-Factor Index of Social Position. New Haven: Yale University Press.
- Holsboer F, Lauer CJ, Schreiber W, Krieg JC (1995): Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. *Neuroendocrinology* 62:340–347.
- Kirschbaum C, Pirke KM, Hellhammer DH (1993): The 'Trier Social Stress Test'—A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28:76–81.
- Kunugi H, Ida I, Owashi T, Kimura M, Inoue Y, Nakagawa S, Yabana T, Urushibara T, Kanai R, Aihara M, Yuuki N, Otsubo T, Oshima A, Kudo K, Inoue T, Kitaichi Y, Shirakawa O, Isogawa K, Nagayama H, Kamijima K, Nanko S, Kanba S, Higuchi T, Mikuni M (2006): Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitary-adrenal (HPA) axis abnormalities in major depressive episode: A multicenter study. *Neuropsychopharmacology* 31:212–220.
- Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL, Meaney MJ (1998): Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci* 1:69–73.
- Makino S, Gold PW, Schulkin J (1994): Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Res* 640:105–112.
- Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003): An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 19:1233–1239.
- Maldjian JA, Laurienti PJ, Burdette JH (2004): Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *Neuroimage* 21:450–455.
- Matsunaga H, Sarai M (2000): Low-dose (0.5 mg) DST in manic and major depressive episodes: In relation to the severity of symptoms. *Seishin Shinkeigaku Zasshi* 102:367–398.
- Matsuoka K, Uno M, Kasai K, Koyama K, Kim Y (2006): Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of National Adult Reading Test. *Psychiatry Clin Neurosci* 60:332–339.
- Mechelli A, Friston KJ, Frackowiak RS, Price CJ (2005): Structural covariance in the human cortex. *J Neurosci* 25:8303–8310.

- Miller GE, Chen E, Zhou ES (2007): If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenal axis in humans. *Psychol Bull* 133:25–45.
- Mollica RF, Caspi-Yavin Y, Lavelle J (1996): The Harvard Trauma Questionnaire (HTQ) Manual: Cambodian, Laotian, and Vietnamese Versions *Torture* 6:19–42.
- Mollica RF, Sarajlic N, Chernoff M, Lavelle J, Vukovic IS, Massagli MP (2001): Longitudinal study of psychiatric symptoms, disability, mortality, and emigration among Bosnian refugees. *JAMA* 286:546–554.
- Morris RC, Evendon JL, Sahakian BJ, Robbins TW (1987): Computer-aided assessment of dementia: Comparative studies of neuropsychological deficits in Alzheimer-type dementia and Parkinson's disease. In: Stahl SM, Iversen SD, Goodman EC, editors. *Cognitive Neurochemistry*. Oxford, United Kingdom: Oxford University Press. pp 21–36.
- Murphy E, Brewin CR, Silka L (1997): The assessment of parenting using the parental bonding instrument: Two or three factors? *Psychol Med* 27:333–341.
- Nelson HE (1982): National Adult Reading Test (NART). Windsor: NFER-Nelson.
- O'Brien JT, Ames D, Schweitzer I, Colman P, Desmond P, Tress B (1996): Clinical and magnetic resonance imaging correlates of hypothalamic-pituitary-adrenal axis function in depression and Alzheimer's disease. *Br J Psychiatry* 168:679–687.
- Oldfield RC (1971): The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9:97–113.
- Pariante CM, Miller AH (2001): Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. *Biol Psychiatry* 49:391–404.
- Parker G, Tupling H, Brown LB (1979): A parental bonding instrument. *Br J Med Psychol* 52:1–10.
- Parker G, Hadzi-Pavlovic D (1992): Parental representations of melancholic and non-melancholic depressives: Examining for specificity to depressive type and evidence for additive effects. *Psychol Med* 22:657–665.
- Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J (2005): Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. *Neurosci Biobehav Rev* 29:649–674.
- Sapolsky RM (1999): Glucocorticoids, stress, and their adverse neurological effects: Relevance to aging. *Exp Geront* 34:721–732.
- Schubert MI, Kalisch R, Sotiropoulos I, Catania C, Sousa N, Almeida OF, Auer DP (2008): Effects of altered corticosteroid milieu on rat hippocampal neurochemistry and structure- an in vivo magnetic resonance spectroscopy and imaging study. *J Psychiatr Res* 42:902–912.
- Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B (1997): Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med* 27:951–959.
- Swaab DF, Bao A, Lucassen PJ (2005): The stress system in the human brain in depression and neurodegeneration. *Age Res Rev* 4:141–194.
- Tyrka AR, Price LH, Gelernter J, Schepker C, Anderson GM, Carpenter LL (2009): Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: Effects on hypothalamic-pituitary-adrenal axis reactivity. *Biol Psychiatry* 66:681–685.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15:273–289.
- Uno H, Eisele S, Sakai A, Shelton S, Baker E, DeJesus O, Holden J (1994): Neurotoxicity of glucocorticoids in the primate brain. *Horm Behav* 28:336–348.
- Woon FL, Hedges DW (2008): Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: A meta-analysis. *Hippocampus* 18:729–736.
- Yehuda R, Lowy MT, Southwick SM, Shaffer D, Giller EL Jr (1991): Lymphocyte glucocorticoid receptor number in post-traumatic stress disorder. *Am J Psychiatry* 148:499–504.
- Yehuda R, Southwick SM, Krystal JH, Bremner D, Charney DS, Mason JW (1993): Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *Am J Psychiatry* 150:83–86.
- Zung WWK (1965): A self-rating depression scale. *Arch Gen Psychiatry* 12:63–70.