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Long-term effects of early parental loss due to divorce on the HPA axis

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Abstract

We investigated the long-term effects of divorce and early separation from one parent on HPA axis reactivity, in young adults without psychopathology. Participants were 44 young subjects, 22 whose parents divorced before they reached age 10, and 22 controls. Psychiatric symptomatology was measured with the Brief Symptom Inventory (BSI), family perceived stress by the Dyadic Adjustment Scale (DAS), and bonding by the Parental Bonding Instrument (PBI). Assessment of HPA axis function included baseline morning cortisol and ACTH and cortisol response to a CRH stimulation test.

No baseline or stimulated group differences were observed for ACTH. Cortisol levels were consistently but insignificantly lower in the divorce group throughout the CRH stimulation reaching statistical significance only at 5 min ($p<0.03$). Group by time effect reached a trend level ($p<0.06$). A correlation was found between psychiatric symptomatology and PBI scores; however, both parameters did not correlate with HPA axis activity. A significant correlation was found between DAS scores and ACTH. A regression model revealed a contributing effect for both family stress and child–parent bonding to stimulated ACTH levels. These preliminary findings suggest that even in the absence of adult psychopathology, a history of childhood separation from one parent due to divorce may lead to detectable, albeit mild, long-term alterations in HPA axis activity. Furthermore, they suggest that level of stress at home and parental bonding are important determinants of this effect. It is likely that divorce has significant and sustained effects on children's HPA axis only in the context of a traumatic separation.

Keywords: Cortisol; Corticotropin releasing hormone; Divorce; HPA axis; Separation; Psychopathology

Introduction

The significance of early negative life events and especially the loss of a significant figure as possible etiological factors in the development of subsequent adult psychopathology has been subject of much speculation and theory for years (Bowlby, 1963). In many studies, an association was reported between EPL due to death (Perris et al., 1986), or other reasons (Dennehy, 1966; Faravelli et al., 1986; Tennant et al., 1982) and the development of affective disorders. Early work suggested a higher incidence of parental death in the history of depressed individuals while later work on this issue was less conclusive (Lloyd, 1980) yet suggested that the loss of the mother either but death or separation may be more significant then the loss of the father (Brown et al., 1997; Kendler et al., 1992; Roy, 1985). These studies are also inconclusive about the significance of the age of loss. Degree of coping with EPL is an important moderator of future psychopathology as demonstrated by Breier et al. (1988) who reported that psychological suffering and the success or lack of coping with the loss of a parent was predictive of future psychopathology.

A large case control study found the incidence of EPL before the age of 17 due to death or separation to be almost 4-fold greater in hospitalized patients suffering from major depression compared to a control group. Moreover, this difference increased to 11-fold when EPL occurred at an age of under 9, and divorce as a cause for separation was found to be more significant than separation due to death of a parent (Agid et al., 1999). The number of studies looking at divorce as the cause for child–parent separation is small. In cases of divorce, compounded onto the need to cope with a "loss" of a parent, research has shown

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that divorce and life in a single parent family make the child more prone to exposure to stressful life events (Amato and Keith, 1991; Hetherington et al., 1985) and that inadequate parenthood may increase the incidence of depressive illness in adulthood (Parker, 1983). A study in Israeli adolescents whose parents have divorced demonstrated higher levels of psychopathology, and a correlation was found between the attachment pattern with their parents and psychiatric symptoms (Canetti et al., 2000). In fact, there is a growing body of literature showing that there may be a considerable difference between long-term effects of bereavement and divorce, and that the impact of divorce is potentially more complicated to adequately assess due to a multitude of potential environmental moderating factors involved in the process (Luecken and Appelhans, 2005; Mack, 2001).

Abnormal function of the Hypothalamic–Pituitary–Adrenal axis (HPA axis) has been well documented in a number of major psychiatric syndromes such as major depression and posttraumatic stress disorder. Hypercortisolemia, a condition that is associated with stress (Kalin and Takahashi, 1988) has been reported to induce glucocorticoid receptor changes and a reduction in hippocampus volume in animal models (Herman et al., 1995; Lopez et al., 1998; Sapolsky, 1996) and in humans (Heim and Nemeroff, 1999; Sheline et al., 1996). It has been suggested that early negative life events may cause hypercortisolemia, which in turn may cause damage to the developing brain during critical periods of development (Gunnar, 1998). In babies, there is a period of decreased reactivity of the HPA axis (Gunnar et al., 1996; Suchecki et al., 1993), which has been found to be dependent on the quality of the baby-parent relationship (Nachmias et al., 1996). Furthermore, in a number of animal models, states of maternal deprivation caused long-term changes of the HPA axis demonstrating a pattern of hyperstimulation (Ladd et al., 1996; Plotsky and Meaney, 1993). Findings in non-human primates exposed to deprivation have not been consistent, with some showing increased (Coplan et al., 1996; Fahlke et al., 2000) and others showing decreased (Clarke, 1993) basal and stress-induced cortisol levels, or decreased morning cortisol levels in monkeys exposed to maternal separation (Dettling et al., 2002). In humans, while a number of studies have demonstrated long-term effects of childhood maltreatment and sexual abuse on the HPA axis, mainly showing increased stress reactivity (De Bellis et al., 1994; Heim et al., 2000, 2001), only a few studies have focused on the effects of parental loss or separation on the HPA axis. In children, studies have been contradictory, some reporting increased morning cortisol in adopted children (Gunnar et al., 2001) and elevated diurnal cortisol secretion in institutionalized children (Kaufman, 1991), and others showing lower morning cortisol in socially deprived children (Carlson and Earls, 1997). In adults with EPL due to parental death, Luecken reported that increased cortisol responses during a psychological stressor were moderated by the quality of the parent-child relationship (Luecken, 2000; Luecken and Appelhans, 2006). Furthermore, whereas, Breier et al. (1988) have reported elevated afternoon cortisol plasma levels only in adults with EPL who had a history of psychopathology, recently, elevated salivary cortisol levels throughout the day but especially in the morning, were reported in adult men who experienced parental death, regardless of psychopathology (Nicolson, 2004). However, a recent study in healthy young adults with EPL (death or separation), found decreased morning salivary cortisol levels (Meinlschmidt and Heim, 2005), while another study did not find a differential cortisol response in adults exposed to various childhood traumatic events (Otte et al., 2005). In the case of divorce as an early life adverse event, there is no literature regarding the long-term effects of this stressor on the HPA axis, though it has been proposed that HPA axis dysregulations may play an important role in the development of mood and behavioral maladjustment to the consequences of divorce (Troxel and Matthews, 2004).

The design of the current study was based on the hypothesis that if EPL indeed causes permanent alterations in the HPA axis, these alterations will precede and may mediate subsequent psychopathology. If this hypothesis is correct, such alterations in the HPA axis are expected to be present even in the absence of psychiatric symptoms (in contrast to the previously described Breier study). To test this hypothesis we studied the long-term effects of childhood parental separation due to divorce on the HPA axis, using the Corticotropin Releasing Hormone (CRH) test, in a young adult population without DSM-IV axis I psychopathology, whose parents divorced (with one of them leaving home) before the subject turned 10 years of age. These criteria were selected to separate the effect of early stress due to divorce from that of current mental condition on the HPA axis. Our main hypotheses were: (a) divorce in early childhood would have long-term effects on the HPA axis in healthy young adults in the form of lower or normal basal cortisol levels, but an increased HPA axis response to CRH stimulation, (b) the effect of divorce on HPA axis response to CRH stimulation will be moderated by level of stress at home prior to the divorce and by the quality of bonding between the child and each one of the parents during childhood.

Materials and methods

Subjects

The separation group comprised of 22 healthy subjects, aged 21–25 whose parents had divorced (with one parent leaving home permanently) before the subject had turned 10 (mean age at divorce=4.6±2.9), and 22 comparison subjects matched on age and sex, who grew up with two married parents at least until age 18. All subjects were recruited by advertisements posted at two university campuses. Interested subjects were screened by phone to exclude those with significant physical or mental illness including brain trauma and alcohol or drug abuse. Also excluded were night shift workers, those whose families have immigrated to Israel before the age of 10, and those who had a deceased parent. The comparison group consisted of volunteers matched for age and sex who grew up with two married parents at least until age 18. All subjects gave written informed consent after they received a detailed explanation regarding the study, which was approved by the hospital's IRB. All participants went through a structured clinical diagnostic interview for DSM-IV (SCID-Hebrew version) (Shalev et al., 1994) to exclude past and present axis I diagnoses. Participants were interviewed regarding their personal demographic details, past traumatic life events (by the Trauma History Questionnaire), personal and family physical and mental disorders, and menstrual history and contraception methods for the women. Exclusion criteria were any past or present axis I disorder, serious physical illness, a past trauma such as a physical

or sexual assault or any life threatening situation, or an irregular menstrual cycle. A total of 6 subjects were excluded from participating in the study, four due to psychiatric diagnoses and 2 due to a major past trauma.

A total of 44 subjects participated in the study, 22 from each group. Table 1 presents basic socio-demographic data on the two groups. As can be seen, the two groups were well matched on all the socio-demographic variables.

Psychometric measures

To measure current psychopathology we used the Brief Symptom Inventory (BSI) (Derogatis and Spencer, 1982). This is a self-administered symptom list that includes 9 different subscales-somatization, obsessive–compulsive symptoms, interpersonal sensitivity, depression, anxiety, hostility, phobia, paranoia, psychosis, and a total score. We used the Hebrew version validated by Canetti et al. (1997) with Cronbach's alpha scores of 0.7–0.97. Because some studies have suggested that levels of stress at home prior to the divorce may be a better predictor of the child's long-term outcome than the divorce itself, we used two instruments to control for the potential moderating effects of level of stress at home prior to the divorce: (1) The Parental Bonding Instrument (PBI) (Gamsa, 1987; Parker, 1983), a self-administered questionnaire that describes the recollection of the parent's attitude towards the subject before the age of 16. The quality of the bonding is described using two subscales for "control" and "care". The Hebrew version used was validated by Canetti (Canetti et al., 1997) with Cronbach's alpha scores of 0.89–0.94. (2) The Dyadic Adjustment Scale (DAS) (Spanier, 1976), a self-administered questionnaire that describes the recollection of the level of conflict between the parents before the divorce. It is divided into 4 subscales assessing dyadic agreement, expression of emotion and fondness, satisfaction, and unity in the marriage.

CRH stimulation test

A standard CRH stimulation test was applied as it has been shown to be sensitive to the effects of early-life adversity in previous studies. All subjects were administered the test at 07:00 after an 8 h fast. The women performed the test during the early follicular phase of their menstrual cycle (first 7 days after onset of menses). A venous catheter was placed in the antecubital fossa followed by a rest period of 45 min. A baseline blood sample for ACTH and cortisol was drawn at −5 min. hCRH (Ferring Pharmaceutical, GmbH CRH) was administered at a dose of 1 μg/kg at time 0, following which blood samples were drawn at 5, 15, 30, 60, and 90 min thereafter. The samples for ACTH were immediately placed on ice and the samples for cortisol were kept at room temperature. All blood samples were then centrifuged and kept frozen at −70 °C until analysis. ACTH was analyzed using an Immulite Analyser (DPC, LA, USA) that uses the chemiluminescent technique based on sequential immunometric assay. Cortisol was analyzed by the same instrument using competitive immunoassay. For ACTH the interassay reliability was 7.9% and the intraassay reliability was 9.6%. The interassay reliability for cortisol was 13.9% with an intraassay reliability of 9%.

Statistical analysis

The sample size for this study was determined by using a power calculation that resulted in a sample size of 20 participants in each group. The criterion for significance (alpha) has been set at 0.050. With the proposed sample size of 20 and 20 for the two groups, the study will have a power of 87% to yield a statistically significant result. This computation is based on previous human studies using the CRH test which found an average change of 30% in the cortisol or ACTH area under the curve (AUC). This effect was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance. It is also assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research. The degree of matching between the two groups was assessed using a set of chi-square tests for categorical variables and a set of *t*-tests for continuous variables. The Wilcoxon test was used in variables that were not normally distributed. The study's main hypothesis, regarding the interaction effect of group and time on ACTH and cortisol levels, was tested using a set of two-way ANOVAs for repeated measures. For each hormone (i.e., ACTH and cortisol), two measures were calculated; (1) the total amount of ACTH and cortisol plasma levels as determined by the total area under the curve (total AUC); and, (2) the net effect of CRH administration as defined by the difference between the baseline and post-treatment levels of plasma ACTH and cortisol (netAUC). In order to normalize these measures, we used a log-transformation of them. The second hypothesis, regarding the effect of child–parent bonding and sex on the AUC was assessed using a set of one-way ANOVAs for repeated measures. The degree of association between child–parent bonding and stress at home parameters and the BSI scores was tested using a set of bi-variate Spearman correlations. The second and third hypotheses, regarding the potential moderating effect that dyadic stress at home and child–parent bonding may have on the effect of early divorce on HPA axis was assessed using two sets of hierarchical regression analyses (a separate set for each one of the two moderating variables) with three steps. On the first, only group (i.e., divorce status) was entered as a sole predictor of HPA axis. On the second step, the main effect of dyadic stress or parental bonding was added to that of group. And finally, on the third step, the group by dyadic stress or group by parental bonding interaction terms (which measure the moderating effect) were added to the equation.

Results

First, we examined the degree of matching between the two groups at baseline. Table 2 presents means and standard

Table 1
Demographic details

Variable	Separation group (n=22)	Control group (n=22)	Significance test
Sex (male/female)	n=9/13 (41%/59%)	n=9/13 (41%/59%)	χ^2=0.00
Marital status (single/divorced/married)	(81%/14%/5%)	(100%/0%/0%)	χ^2=5.64
Living with partner (yes/no)	(67%/33%)	(64%/36%)	χ^2=0.04
Religion (Jewish/other/unknown)	(91%/4.5%/4.5%)	(95%/5%/0%)	χ^2=1.02
Ashkenazi/Sephardic/unknown	(81%/6%/13%)	(90%/10%/0%)	χ^2=2.83
Country of birth (Israel/other)	(64%/36%)	(62%/38%)	χ^2=0.01
Education (high school/B.A./M.A.)	(4%/82%/14%)	(0%/91%/9%)	χ^2=1.37
Military service (full/partia/exempt/student)	(55%/9%/18%/18%)	(36%/14%/23%/27%)	χ^2=1.51
Socioeconomic status (below average/average/above average)	(24%/62%/14%)	(14%/82%/4%)	χ^2=2.28
Living status (independent/family/other)	(36.5%/27%/36.5%)	(41%/32%/27%)	χ^2=0.42
Shift work (yes/no)	(55%/45%)	(68%/32%)	χ^2=0.86
Age (years)	23 (1.4)	23.4 (1.5)	F=0.74
Age at divorce (years)	4.6 (2.9)	–	–
Weight (kg)	69.8 (14.8)	67.9 (11.4)	F=0.47
Number of rooms in parent's home	3.7 (0.9)	4.2 (1.1)	F=2.35

None of the tests reached significance levels.

Table 2
BSI symptom scores according to subscales

Symptom subscale	Separation group mean (SD)	Control group mean (SD)	Wilcoxon
Somatization	0.20 (0.36)	0.07 (0.13)	0.92
Obsessive–compulsive	0.52 (0.55)	0.37 (0.44)	0.84
Interpersonal sensitivity	0.66 (0.84)	0.38 (0.39)	0.70
Depression	0.58 (0.68)	0.39 (0.49)	0.48
Anxiety	1.36 (0.79)	1.09 (0.29)	1.27
Hostility	0.51 (0.56)	0.26 (0.35)	1.69
Phobia	0.42 (0.68)	0.19 (0.34)	1.10
Paranoia	0.53 (0.85)	0.33 (0.36)	0.01
Psychoticism	0.36 (0.56)	0.27 (0.43)	0.47
Total score	0.46 (0.56)	0.27 (0.27)	0.82

* = $p<0.05$.

deviations of symptom levels at baseline. As can be seen, baseline symptom levels, as assessed with the BSI, were rather low in both groups. This is not surprising considering that both groups were screened for Axis I diagnoses. As could be expected, subscale scores were slightly higher, though not significantly so, in the study (divorce) group across all the clinical measures. The only exception to this general rule was "hostility" which was significantly higher in the divorce group; however, this difference did not remain significant after adjusting the *p*-value for multiple comparisons using the Bonferroni correction.

Next, we tested the first hypothesis regarding an increased response of the HPA axis to CRH stimulation in the divorce group. Fig. 1 presents the change over time in ACTH and cortisol levels in the 2 groups. A set of two-way ANOVAs revealed significant time effects for mean plasma ACTH response to an iv bolus of hCRH ($F_{5,37}=19.5$, $p<0.01$) and for cortisol ($F_{5,39}=16.2$, $p<0.01$).

While the group by time interaction effect was non-significant for ACTH ($F_{5,39}=0.5$, $p=0.68$), it approached significance for cortisol ($F_{5,39}=1.94$, $p=0.06$). Similarly, while the effect size (using Cohen's conventions, Cohen, 1988) for ACTH was small (ES=0.20) the effect size for cortisol was medium (ES=−0.41). Group means showed significantly lower cortisol plasma levels at 5 min after CRH injection for the separation group compared to the control group ($F_{1,43}=5.3$, $p=0.025$) (Fig. 1). This overall pattern of results remains unchanged after repeating these analyses with ACTH levels at baseline as a covariate. Similarly, the results remained unchanged when the data wee reanalyzed by gender. In summary, while the lower cortisol level found in the separation group was as hypothesized, activation of the HPA axis subsequent to CRH challenge was negative for ACTH and only at a strong trend level for cortisol. Consequently, contrary to our hypothesis, the difference between HPA axis activation in the two groups was not significant.

No significant inter-group differences were found for either the calculated total and net AUC for ACTH and cortisol between the study and control group. A significant main effect of sex (female>male) on total AUC was found for both ACTH ($F_{1,43}=14.4$, $p<0.01$) and cortisol ($F_{1,43}=12.1$, $p<0.01$) but no significant total or net AUC by sex by group interaction was detected for either hormone.

Next, we tested the second hypothesis regarding the moderating role of level of stress at home on the effect of divorce on the HPA axis. To test this hypothesis, we reanalyzed our data with parental conflict (assessed with the DAS) as an interacting variable. First, we looked at the relationship between DAS and ACTH AUC and cortisol AUC. The Pearson correlation between these two variables was significant for ACTH AUC only ($r=-0.32$. $p<0.05$). A particularly strong correlation was found between ACTH levels and the measure of dyadic agreement. Next, we divided the group into high-conflict versus low-conflict using the median DAS score as a cut-off point. As would be expected, a chi-square analysis revealed a strong association between divorce status and level of parental conflict ($\chi^2=23.3$; $p<0.01$). Finally, we conducted a hierarchical regression analysis with three steps. On the first, group (i.e., divorce status) only was entered. The obtained R-squares were 0.040 for ACTH and 0.037 for cortisol. On the second step, the effect of stress at home (DAS) was added to that of group. The resulting *R*-squares were 0.063 for ACTH and 0.038 for

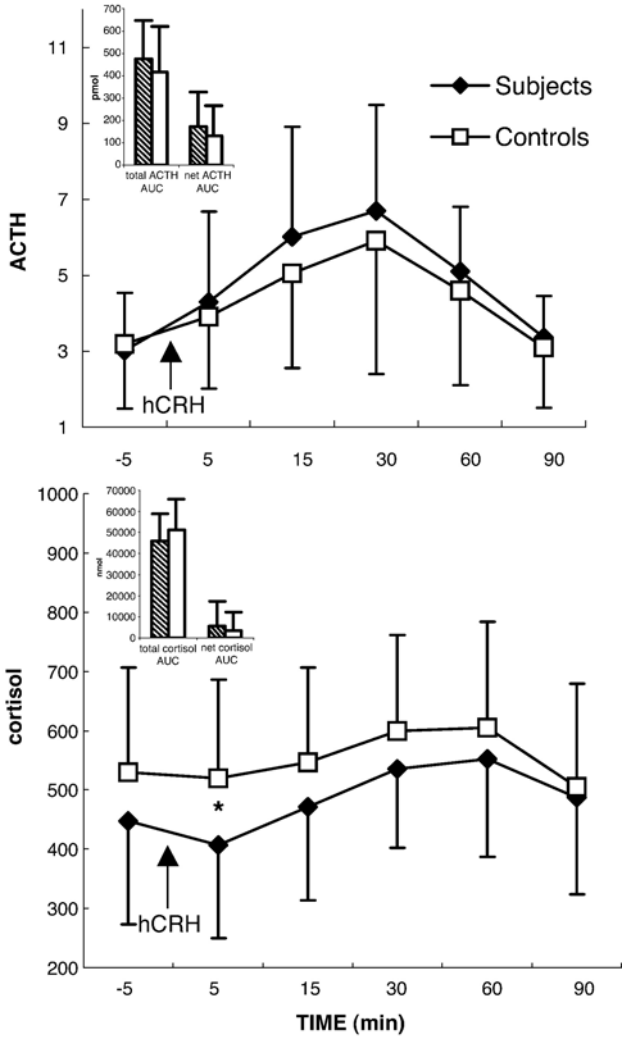

Fig. 1. Plasma ACTH and cortisol levels after stimulation by CRH (mean+SD). Inset—total (left bars) and net (right bars) area under the curve (striped—subjects, white—controls). *Subjects vs. Controls 5 min after CRH injection; $p=0.025$.

cortisol. Lastly, on the third step, the group by stress interaction term was added to the equation. The obtained *R*-squares were 0.065 for ACTH and 0.038 for cortisol. These results do not provide support for a moderating role of stress at home on the effect of divorce on HPA axis. However, at least for ACTH, they do suggest that stress at home has an important additive effect over and above that of divorce alone. In fact, for ACTH, stress at home was the best single predictor (*R*-square = 0.062).

Finally, we examined the potential moderating role of parental care and controlling patterns as measured by the PBI on the effect of divorce on HPA axis. First, we looked at the relationship between the PBI and ACTH AUC and cortisol AUC. No association was found between the Parental Care and Control subscales and total or net AUC for neither ACTH (r range = 0.02 to 0.30; p range = 0.92 to 0.06) nor cortisol (r range = 0.02 to −.19; p range = 0.90 to 0.23).

Second, we examined the effect of parental caring and controlling patterns on clinical symptoms in adulthood. Table 3 presents means and standard deviations on the PBI in the two groups. As can be seen, while a caring bond with the mother (care mother) is described in equal terms in both groups, in the early separation group there was a trend for the mother to be perceived as more controlling. The relationship with the father in the separation group was perceived as less caring and more controlling. For the whole group, a significant negative correlation was found between the care-father subscale of the PBI and total BSI score ($r = -0.38$, $p < 0.01$) and a trend for significance between the control-mother subscale and total BSI scores ($r = 0.28$, $p < 0.06$).

Lastly, we conducted a hierarchical regression analysis with three steps. On the first, group (i.e., divorce status) only was entered. The obtained *R*-squares were 0.047 for ACTH and 0.030 for cortisol. On the second step, the effect of mother attachment and father attachment was added to that of group. The resulting *R*-squares were 0.24 for ACTH and 0.07 for cortisol. On the third step, the group by mother and father interaction terms were added to the equation. The obtained *R*-squares were 0.26 for ACTH and 0.10 for cortisol. These results do not provide support for a moderating role of parental attachment on the effect of divorce on HPA axis. However, at least for ACTH, they do suggest that parental attachment, specifically maternal one, has an important additive effect over and above that of divorce alone. In fact, in this model for ACTH, maternal bonding was the best single predictor (*R*-square = 0.17).

Table 3
Bonding pattern with parents; the Parental Bonding Instrument (PBI mother/father)

Parameters	Separation group mean (SD)	Control group mean (SD)	Wilcoxon
Care-mother	25.64 (4.85)	26.82 (4.99)	0.50
Control-mother	11.95 (7.85)	7.72 (6.21)	3.63ϕ
Care-father	14.55 (7.97)	23.09 (6.65)	10.13**
Control-father	13.52 (6.96)	6.91 (8.06)	7.36**

ϕ = $p < 0.06$.
** = $p < 0.01$.

Discussion

Long-term effects of early life events on the HPA axis have been demonstrated in animal models of maternal separation (Plotsky and Meaney, 1993; Ladd et al., 2000). In humans, relatively few published studies were designed to look at the long-term effects of early adverse life events, most of which were focused on long-term effects secondary to childhood physical or sexual abuse (De Bellis et al., 1994; Heim et al., 2001) and parental separation in the presence of mental disorder (Breier et al., 1988; Petitto et al., 1992). We used the CRH stimulation test to assess the long-term effects of a "milder" childhood adverse event, parental separation by divorce, on the HPA axis in the absence of significant mental illness.

Our findings provide partial support for the presence of a long-lasting effect of early separation due to divorce on the HPA axis. While both baseline and stimulated cortisol levels were consistently lower in the separation group, these did not reach statistical significance except for the 5-min post-CRH stimulation time point. As the effect of the CRH stimulation on cortisol is not expected within this short time period, this time point can be argued to reflect baseline levels; however, this conclusion cannot be definitely made. Thus, while inconclusive, our finding provides some support in favor of recent publications reporting either lower cortisol levels (Meinlschmidt and Heim, 2005) or no differences (Otte et al., 2005) in adults with early parental separation or childhood traumatic events, and is in contradiction to the report by Nicolson (2004) who reported higher cortisol levels in adult men with parental loss or separation. While our results for stimulated cortisol showed a pattern of increased response in the separation group with a medium effect size, the difference only reached a trend level of significance ($p = 0.06$). As our results are suggestive of possible decreased baseline cortisol levels, and those for stimulated cortisol approach significance, it is possible that a larger sample would have had the power to detect such a difference, that probably exists, but is less dramatic than was expected (type II error). Thus, our results should probably be considered as potentially positive, albeit preliminary. As it is, our results are consistent with studies of subjects with clinical syndromes such as PTSD (Yehuda, 1997) or life-long trauma with or without PTSD (Young and Breslau, 2004), in which increased levels of cortisol were not found, or in women with PTSD who had experienced childhood sexual abuse in which significantly enhanced suppression of plasma cortisol in response to dexamethasone was reported (Stein et al., 1997). Our findings are also partially in line with findings from studies in a group of depression-free women survivors of childhood sexual abuse (Heim et al., 2001) in whom lower baseline and stimulated cortisol levels were reported.

Several factors might explain the fact that our study hypothesis was only partially supported by our results. First, to test our hypothesis that permanent alterations in the HPA axis following childhood trauma actually precede and possibly mediate subsequent psychopathology, we excluded from our sample subjects with a history of other significant traumas, past or present psychopathology and family history of mental

disorder. This is quite different from most previous studies that did include subjects with a history of psychopathology (Breier et al., 1988; Heim et al., 2001; Petitto et al., 1992). Whereas children of divorced parents have been reported to have more psychopathology than controls (Amato and Keith, 1991; Hetherington and Stanley Hagan, 1999), our subjects had an exceptionally low BSI score. This may be due to two main reasons: First, the recruitment method of advertisements in university campuses, which may have resulted in self-selection of highly functioning asymptomatic individuals, and, second, the exclusion of individuals with formal axis I diagnoses ($n=4$). Thus, a possible limitation of our approach is the selection of a population who may represent a group of "super-normal" subjects who coped with their parents' divorce in an exceptionally successful manner, and may thus actually represent a selected subgroup possessing unknown "resiliency factors". Alternatively, major HPA axis abnormalities may only reflect existing psychopathology rather than a preexisting, persisting, abnormal physiological liability due to the early childhood traumatic event that mediates vulnerability to stress and subsequent psychopathology as hypothesized.

Second, contrary to most studies regarding the effects of early childhood physical or sexual abuse on the HPA axis that were conducted on an exclusively female population, we studied both males and females. We found higher baseline and stimulated cortisol secretion in the female subjects, a finding that is consistent with previous reports (Young, 1996). However, an interaction between group and cortisol or ACTH secretion was not found for either males or females. Due to the relatively small number of males in this study ($n=18$), any conclusion about the effect of sex on the long-term effects of divorce on the HPA axis is limited and a replication with larger numbers is in place.

Many studies have reported on the long-term effects of maternal deprivation on the HPA axis in animal models and in children as well (Coplan et al., 1996; Gunnar, 1998; Ladd et al., 2000; Plotsky and Meaney, 1993). This effect may be due to the effect of high levels of corticosteroids on the developing brain (Bremner, 1999). In the present study we chose divorce of the parents and separation from at least one of them as the stressful event. Subsequent to the divorce, our subjects remained with one parent, usually the mother, who is still, in most cases, the primary caretaker. This is in contrast to most animal models of separation in which the animal was separated from the mother and remained on its own. Furthermore, we could not control for a possible "buffering effect" of a potential non-biological father figure in the child's life after the divorce. In the future, a possible design to overcome this limitation would be to study the effects of separation at an early age from both parents. The mean age for our subjects during the divorce was 4.6 years, a relatively old age in terms of separation. It is possible that had we limited our sample to cases in which the divorce occurred when the children were all infants under 3 years of age or younger, the effect on the HPA axis would have been more pronounced. Although studies of sexual abuse in childhood in which HPA axis alterations have been demonstrated were conducted on girls of older age (Heim et al., 2000, 2001), such an event can be considered as far more traumatic than divorce and also it is assumable that in such families other acts of violence or neglect have preceded the event of sexual assault at a younger age.

Furthermore, there is some evidence that "moderately stressful" early life experiences may actually serve as a "resiliency factor" to subsequent stressors (Parker et al., 2004), and that "stress inoculation" in mild stress may be more relevant in non-human primates than "maternal modulation" (Parker et al., 2006). Thus, it is possible that the "traumatic event" chosen for this study, divorce of parents and separation from one of them, is not a sufficiently strong stressor to induce, in itself, permanent HPA axis abnormalities. Further in this respect, the design of this study did not allow us to consider genetic predisposition factors that probably play a role in determining vulnerability to early life stressors and HPA axis responses. It is probable that child, family, and genetic factors all play a role in the development of resilience to childhood stress due to parental loss (Lin et al., 2004).

Finally, a technical design limitation that may have confounded our results is the fact that the CRH challenge was performed in the early morning rather than in the afternoon when endogenous HPA axis activity is low. While CRH challenges have been done successfully in the morning hours as well, they are less sensitive and it would have been a definite study design advantage, possibly leading to more robust findings, had the pharmacological challenge been performed in the late afternoon. Furthermore, we used the CRH stimulation test, which is a pharmacological test of HPA activity, and it is certainly possible that additional evaluations of unstimulated conditions of the HPA axis (such as urinary free cortisol) may have unraveled alterations of the HPA axis in the study group.

Divorce is a common phenomenon in modern western cultures and despite obvious difficulties; most children of divorced parents grow up to be adequately functioning adults (Chase-Lnasdale et al., 1995; Hetherington, 1993). In fact, if as a consequence of the divorce, a stressful environment is replaced by a more harmonious one, the child may even benefit from the divorce (Amato et al., 1995). Thus, it is reasonable to assume that it is not the divorce itself that is detrimental to the child's mental health but rather the variety of interactions and events that precede and follow the divorce that have the actual impact on the child's emotional outcome. Similarly, it would be erroneous to assume that children whose parents never separated grew up in a stress-free environment. To control for these potential confounds, we tried to look at the tension level at home prior to divorce and the bonding pattern between parents and child as a possible contributing factors between the divorce and subsequent development of psychopathology. The data we present on the amount of stress in these families prior to the divorce provide partial support for the notion that such contributing factors are in fact crucial for the development of psychopathology. As one might expect, we found a marked association between divorce and level of tension at home during childhood, but more importantly, we found a significant correlation between ACTH levels and measures of tension at home during childhood. Moreover, tension at home and the quality of child–parent bonding were the best predictors of

ACTH levels in this study. This finding is consistent with a recent report of a negative correlation between cortisol responses to a CRH challenge and chronic family stress (Ronsaville et al., 2006). Furthermore, while correlations between child–parent bonding patterns and HPA axis measures were not significant, we did find that a caring relationship with the father was associated with less psychopathology while a controlling relationship with the mother was associated (at a trend level) with more psychopathology even in this population of healthy subjects. From the literature we know that adults who have experienced inadequate parenting in their childhood show a compromised ability for coping with stressful life events in their adulthood (Rutter, 1999). Among adults who have lost a parent during childhood, those who developed psychopathology, reported less support from the living parent compared to those without psychopathology (Breier et al., 1988). In another study, adolescents of divorced parents described them as more controlling and intrusive and less caring and supportive, an interpersonal pattern that was found to be related to psychiatric symptomatology (Canetti et al., 2000). In conclusion of this part of the study, we provide limited support for an association between early stress within the family and HPA axis measures (ACTH) in adulthood, and some further support for an association between early stress and bonding patterns and subsequent psychopathology.

In summary, while the mixed results of this study must be interpreted with caution and regarded as preliminary, it can be concluded that in the absence of adult psychopathology, EPL due to divorce may result in long-term alterations in HPA axis activity. Furthermore, the results indicate that stress levels at home before the divorce and the perceived quality of child–parent bonding are important determinants of the long-term effects of divorce on the HPA axis. Thus, it is likely that divorce might have significant and sustained effects on children's HPA axis only in the context of a traumatic separation. The effect of divorce, a relatively common childhood negative life event, on the HPA axis in adults with psychopathology should be further explored in more heterogeneous populations. Furthermore, it is important to further characterize children who remain resilient to the effects of divorce on the HPA axis and subsequent adult psychopathology.

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