CHAPTER 2

Diurnal Cortisol Patterns and Stress Reactivity in Child Holocaust Survivors

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Manuscript submitted for publication
Abstract

Late-life implications of early traumatic stress for the adreno-cortical system were examined in a sample of 133 child survivors of the Holocaust, who were subjected to Nazi persecution during infancy. Method: In a non-convenience sample of child survivors, born between 1935-1944, basal circadian cortisol release and cortisol reactivity to a stressor were assessed. Results: Age, parental loss during the Holocaust, current depression, PTSD and physical illness were not associated with differences in basal diurnal cortical levels. Neuro-endocrine effects, however, were found in stress reactivity through elevated cortical levels in male respondents in the youngest age group (born 1941-1945), and in male respondents suffering from PTSD-related functional impairment. Conclusion: The youngest survivors of Nazi persecution show late-life effects of traumatic stress during early childhood, evidenced by the early onset of differential neuro-endocrine pathways to stress-regulating strategies.
Introduction

Over the last twenty years Jewish child survivors of the Nazi Holocaust have become identified as a subgroup of Holocaust survivors with specific needs, differing from those of survivors who were adults during the Holocaust (Kestenberg & Brenner, 1996; Krell, 1985). They have become the focus of an increasing number of studies on the effects of childhood deprivation on coping with later-life challenges (Keilson, 1992; Moskovitz, 1985; Moskovitz & Krell, 1990; Tauber, 1996). Today, there exists an impressive body of descriptive clinical case studies, as well as empirical studies. On this basis, Dasberg (2001) identified an "adult child survivor syndrome", "a price paid through lifelong [post-Holocaust] symptoms in different combinations, intensities, and courses over time".

Several studies have been carried out over the years among various groups of child Holocaust survivors with matched controls who did not suffer Nazi persecution. Samples were recruited in part from treatment–seeking populations, and through affiliates of Holocaust interest groups (Amir & Lev-Wiesel, 2003; Brom, Durst & Aghassy, 2002; Cohen, Dekel, Salomon & Lavie, 2003). Others studies included randomized, non-convenience, non-treatment-seeking samples of Holocaust child survivors (Cohen, Brom & Dasberg, 2001; Sagi, Van IJzendoorn, Joels, & Scharf, 2002). The overall outcomes of these studies show significantly more traumatic stress with child Holocaust survivor samples in general as compared to controls, whereas treatment-seeking Holocaust survivors show significantly more severe post-traumatic stress symptomatology.

As a rule, studies of child survivors of the Holocaust include persons born between 1927 and 1945, which means that on Capitulation Day, May 8th 1945, they were aged from several months to 18 years. Interestingly, only Keilson (1992) designed a study taking into account differences in developmental age at the time of persecution. In clinical descriptive studies, some authors focused on issues related to the effects of developmental age during the time of persecution (Durst, 2003; Gampel, 1988; Kestenberg, 1988; Kestenberg & Gampel, 1983; Van der Hal, 1996). To our knowledge, no specific, systematic research exists focusing exclusively on effects of Holocaust persecution and its aftermath on the life cycle of the youngest children, born between 1935 and 1944. Aged from several months to 10 years at the end of the Second World War in 1945, many had to cope with the stress of deprivation and violence and want during the very first years of life. The oldest of this group of survivors were born during the first years of the Nazi regime, when, although not in direct life danger, their families endured progressively deteriorating physical, social and economic living circumstances. The younger of these child survivors were born during persecution, when their family units had disintegrated under death threat. Some parents succeeded in keeping
these children alive in concentration and work camps, or while fleeing into forest
or mountain areas, or to the harsh living conditions in Siberia and Uzbekistan.
Other parents separated from their children, intuiting that the chances for surviving
changes were slimmer if they stayed together. They handed them to Christian
families, monasteries, and other institutional care. Infants were left on doorsteps,
hurled out of deportation trains or over ghetto walls, and “smuggled” out of
deportation centers in waste bins and laundry baskets. Care provided to them by
Gentile strangers varied from excellent to abusive in physical, sexual and emotional
ways. Some children stayed during the persecution period with one care provider,
others had to cope with and adapt to several, and sometimes many, caretakers.
After liberation, many of the children suffered social and relational estrangement.
When they survived separated from their families, they were now reunited with
surviving parents they remembered only vaguely, if at all. Others had to cope with
the loss of their murdered parents.

The current study focused specifically on the present day life of these
youngest Holocaust survivors, who are now in their sixties, assessing influences of
early childhood exposure to the traumatic stress of the Holocaust. While
conceptualizing this study, we were aware of the fact that for the youngest
survivors, in particular when born during persecution, the ability of their primary
caregivers to stay fully attuned to their needs of proximity and safety could have
been compromised (Bar-On, Eland, Kleber, Krell, Moore, Sagi, et al., 1998; Siegel,
1999). As a result, we opted to examine the effects of their early childhood
experiences on the level of psycho-physiological functioning, in particular the
adreno-cortical system.

Studies in animals and human infants showed that maternal separation and
loss during infancy may have long-term effect on social adjustment, cognitive
functioning and behavioral responses to stress (Gunnar & Nelson, 1994; Lui et al.,
1997; Sanchez, Ladd & Plotsky, 2001; Sapolsky & Meaney, 1986). Variations in
maternal care also influence responses to stress in offspring by altering the
development of the neural systems that mediate fearfulness (Weaver et al., 2004).
As stress responses are physiological coping responses, they involve several body
regulation systems: the sympathetic nervous system, the neurotransmitter system,
the immune system, and the hypothalamic-pituitary-adrenal (HPA). Cortisol is the
primary hormonal product of the HPA axis, the adreno-cortical system. During
stress the hypothalamus signals the pituitary gland to stimulate the release of
cortisol from the adrenal gland. The function of cortisol is to inactivate other
biological reactions that were mobilized to cope with a stressor. In this sense one
can conceptualize cortisol as an “anti-stress” hormone (Yehuda, 1997).

The production of cortisol follows a circadian rhythm with the highest level
around 30 minutes after morning wake-up. During the day cortisol levels decrease
first sharply, and later on more gradually into the evening. An early morning peak and evening nadir can be observed in children as early as three months of age (Larson, Prudhomme White, Cochran, Donzella & Gunnar, 1998). At the same time, cortisol levels are also sensitive to instant emotional and physical stressors (Kirschbaum & Hellhammer, 1989, 1994). Therefore, superimposed upon the diurnal patterns, cortisol levels are activated by environmental cues relating to threats, unfulfilled expectations, pain, infection or metabolic crises (Glaser, 2000).

The human postnatal HPA axis system is highly responsive to stimulation, even when a diurnal rhythm is still lacking (Goodyer, Park, Netherton & Herbert, 2001). De Weerth, Van Hees & Buitelaar (2003) reported a relation between higher cortisol values during late pregnancy, earlier delivery, and more difficult-to-handle infant behavior, especially during the first seven weeks of life. Gunnar and colleagues studied the developmental changes that occur in the reactivity of the adreno-cortical system during the first years of life. They found shifts in decreasing reactivity of the HPA axis, the first occurring up to three months of life, and a second between 3 and 12 months (Gunnar, Brodersen & Krueger, 1996). Furthermore, they noticed the development of "social buffering", which enables a lower sensitivity of cortisol activity to variations in care quality. They also found evidence that children with negative emotional temperament are most likely to show higher levels of cortisol under less than optimal caring conditions (Gunnar & Donzella, 2002; Nachmias et al., 1996).

In adults, vulnerability induced by adverse experiences in childhood has been associated with altered stress reactivity and altered diurnal cortisol levels, yielding in different studies normal, as well as higher or lower than normal, levels of cortisol (hypo- or hyper-cortisolism) (Heim et al., 2000). Yehuda and colleagues (Yehuda, 1997, 2002; Yehuda, Golier & Kaufman, 2005), however, consistently found lower than normative diurnal cortisol levels in adult Holocaust survivors and their offspring, who also suffered from PTSD.

Gender, as well as age, influences on cortisol reactivity to a stressor were reported in several studies. In 102 healthy subjects between 9 and 76 years, Kudielka, Buske-Kirschbaum, Hellhammer & Kirschbaum, (2004) found that an acute psychological stressor induced significant HPA axis responses in all age groups. While no gender differences appeared in children and younger adults, elderly men showed larger free cortisol responses than elderly women. In another study, cortisol responses to a speech task differed by age (range 43-86 years), with the smallest responses in the oldest age group (Nicolson, Storms, Ponds & Sulon, 1997). In this study, younger men (40-59 years) in particular showed the largest and most prolonged response, while the elderly women (70 years and older) were the least likely to show any response. Wolf, Schommer, Hellhammer, McEwen & Kirschbaum (2001) found that younger women (with a mean age of 24.9 year; SD:
1.2 year) did not show any association of reduced memory performance with strongly induced cortisol increase, but men in the same age range did.

Several studies evaluated the cortisol responses of PTSD-patients with stress induction by means of “traumatic reminders”. Veterans with PTSD evidenced elevated cortisol levels compared to veteran controls without PTSD after a challenge exposure to white noise and combat sounds (Liberzon, Abelson, Flagel, Raz & Young, 1999). Elzinga, Schmahl, Vermetten, Van Dyck & Bremner (2003) found PTSD symptoms highly predictive of cortisol levels in abused women with and without PTSD after they were exposed to personalized trauma scripts. Adult women, victims of early childhood abuse and suffering from depression, showed increased cortisol responses to a cognitive challenge (Heim, Newport, Bonsall, Miller & Nemeroff, 2001).

Against the background of these studies, which document somewhat diverging findings on the influences of early traumatic stress on HPA axis functioning, we assessed the influences of earliest childhood exposure to the traumatic stress of the Holocaust on both diurnal cortisol patterns and cortisol reactivity to a stressor. First, we expected to find that the youngest child survivors, who experienced the Holocaust atrocities at the most critical stage of their lives, would show a deviating diurnal cortisol pattern, related to suffering from PTSD, depression and physical illnesses. Second, we expected the youngest Holocaust child survivors, who also lacked the pre-war experience of a relatively protected family life, to show the most elevated cortisol responses to a stressful challenge, with men showing stronger responses than women.

Method

Participants

Participants were 203 child Holocaust survivors, who were born between 1935 and 1944 in countries occupied by the Nazi regime, and who immigrated to Israel after 1945. A non-convenience sample was created by recruiting through demographic information provided by the Israel Ministry of Interior Affairs, including name, year and country of birth, and date of immigration into Israel. Regulations in Israeli laws concerning invasion of privacy were maintained. Invitations to participate in the study were sent by mail to 410 addresses. In a follow-up telephone call 293 survivors who met our criteria could be reached. Forty-nine survivors refused to participate, while 41 candidates were not available for participation during the time frame of the study. Following the regulations of the Israeli Ministry of Health, all participants signed forms of informed consent after they had received an explanation of the purpose of the study.
We decided to introduce saliva collection procedures for cortisol determinations halfway through our study, resulting in a sub-sample of 133 survivors for whom cortisol data were available. These survivors were on average 65 years old, and 61% were female. For the purpose of analysis, the sample was divided in three age groups: born between 1935-1937 (n = 43), between 1938-1940 (n = 43), and between 1941-1944 (n = 47). Fourteen child survivors (11%) had lost both their parents during the Holocaust, 28 had lost one parent (21%), and in the remaining group (68%) both parents survived.

**Procedures and measurements**

*Cortisol.* Research assistants instructed participants in their home or at the Amcha Center for Holocaust Survivors, according to the preference of the participants. They provided oral and written explanations for taking three saliva samples for basal cortisol measurements during a normal day, the first upon awakening, the second before lunch, and the third before dinner. Instructions included mouth rinsing before sampling, refraining from eating fruit or drinking fruit juice, refraining from smoking for half an hour before sampling, and refraining from drinking alcohol for at least 12 hours before starting the sampling procedures. Respondents were asked to note the exact time when they collected saliva, and to report stressful activities, their state of health, and medications taken during the sampling day. Saliva samples were frozen immediately after collection.

Several days to two weeks later, respondents participated in a stressful task which consisted of completing self-report questionnaires. A more detailed description of this stressor can be found below. Three saliva samples were collected at 20-minute intervals during the procedure, with the first sample taken 20 minutes after the start. After a resting period of 40 minutes, a fourth sample was taken to assess the post-stress cortisol level. All samples were frozen until assayed for cortisol concentration. Research assistants were present during the whole procedure, and were available for emotional support at the time of the stressor, and by phone at any time afterward.

The saliva samples were stored at -20°C until analysis. The samples were analyzed in the laboratories of Trier University (Germany, Department of Clinical and Theoretical Psychobiology).

After thawing, saliva samples were centrifuged at 2000xg for 10 minutes, which resulted in a clear supernatant of low viscosity. Saliva (100 l) was used for duplicate analysis. Cortisol levels were determined by employing a competitive solid-phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFIA). Maxisorb microtiter plates (96-well) (Nunc) were coated with rabbit anti-ovine immunoglobulin. After an incubation period of 48 hours at 4°C plates were washed three times with wash buffer (pH=7.4; containing sodium...
phosphate and Tween-40). The plates were then coated with an ovine anti-cortisol antibody and incubated for 48 hours at 4°C. Synthetic saliva mixed with cortisol in a range of 0-100 nmol/l served as standards. Duplicate samples of standards, controls (saliva pools) and samples were tested. Biotin-conjugated cortisol (50 μl) was added and the non-binding cortisol/biotin-conjugated cortisol was removed after 30 min of incubation by three rounds of washing. Europium-streptavidin (200 μl) was added to each well and enhancement solution (200 μl) was added after 30 min and six rounds of washing (Pharmacia, Freiburg, Germany). Within 15 min on a shaker the enhancement solution induced fluorescence which was detected with a DELFIA-Fluorometer (Wallac, Turku, Finland). A standard curve was generated using a computer-controlled program and the cortisol concentrations of the samples were calculated. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% - 9.0%. Log-transformed cortisol levels were used in the analyses.

Stressor. Through the questionnaire participants were confronted with questions about their Holocaust survival experiences and exposure to other shocking life events: e.g. sexual, physical or emotional abuse, traumatic experiences during the wars and the terrorist attacks in Israel, combat trauma, death of close relatives after the Holocaust, life-threatening illnesses and traffic accidents. In addition, they completed several standard psychological assessment questionnaires. The procedure took ninety minutes on average.

Instruments

Physical health status. Physical health status was assessed by a self-report questionnaire listing eighteen chronic physical illnesses. Respondents were asked to indicate which, if any, illnesses they had suffered during the last month. This questionnaire is widely used in Israeli institutes for socio-demographic research on the aged.

Beck depression inventory for primal care. This seven-item self-report questionnaire is widely used for fast screening for depression in adults. Sensitivity and specificity rates are 82%, slightly lower than the longer version. The seven items pertain to feelings of sadness, discouragement about the future, perceived decrease in self-confidence, a sense of being overly self-critical and a suicidal ideation. Each question is answered on a scale of 0-3 (Beck, Guth, Steer & Ball, 1997). Alpha reliability in the current sample was .75 (n = 198).

Post-traumatic stress diagnostic scale. PTSD functional impairment was assessed by means of the PDS. This 49-item self-report scale assesses DMS-IV symptoms of PTSD. It provides a categorical diagnosis of PTSD, as well as an overall measurement of symptom severity. The instrument showed good internal consistency and test-retest reliability. The test items correspond to DSM-IV
(American Psychiatric Association, 1994) diagnostic criteria for PTSD, indicating satisfactory convergent validity and concurrent validity assessed by self-report measures of depression and anxiety (Foa, Cashman, Jaycox & Perry, 1997). In the current study we used the PTSD F-criterion for functional impairment as a stringent index of PTSD with implications for daily functioning of the participant. The F-criterion, part four of the PDS, ascertains the level of impairment in social, occupational, inter-relational and other important areas of personal functioning. It consists of nine questions requiring a yes-no answer on perceived disturbances in daily functioning during the last month, as a result of a traumatic experience. Internal consistency in the current sample was adequate (alpha .82, \( n = 108 \)).

Holocaust survival experience. In the current study, we interviewed the participants about their age and experiences during persecution, and parental loss as a result of the Holocaust.

Results

Preliminary analyses

Participants in the oldest age group more often lost one or both of their parents during the war, \( F (2, 131) = 3.59, p = .03 \). No difference in physical illness among the groups was found (see Table 1). The three age groups did not differ on depression, in the proportion of female survivors, or in the proportion of participants who used medication.

There was no difference in age among survivors who reported no PTSD functional impairments, those who did experience PTSD functional impairments, and the survivors who did not relate to any traumatic experience as currently disturbing them. Survivors with PTSD functional impairments suffered significantly more physical illnesses and they were significantly more depressed than the other two groups (Table 2). The three groups contained similar proportions of females, and did not differ in the use of medication. Since the group of survivors who did not relate to any traumatic experience as disturbing them did not significantly differ from the group without PTSD functional impairments on any of the variables (see Table 3), the two groups were collapsed in the analyses.

Survivors with more physical illnesses were more depressed, \( r (131) = 0.23, p <0.01 \). Depression scores were significantly higher in the group of survivors who lost both parents (\( M = 4.29, SD = 4.38, n = 14 \)) compared to survivors who lost one parent (\( M = 1.82, SD = 2.36, n = 28 \)) or who lost no parents (\( M = 2.25, SD = 2.35, n = 91 \)) during the Holocaust (\( F (2,130) = 4.44, p = 0.01 \)). Physical health status did not differ for groups with varying parental losses.
Loss of parents, depression, and physical health were not significantly correlated with any of the cortisol measures, i.e. the three diurnal cortisol measurements and the three cortisol stress reactivity measurements. Correlations ranged from $r = -0.18$ ($p = 0.06$, $n = 109$, for the relation between noon cortisol and health) to $r = 0.15$ ($p = 0.10$, $n = 120$, for the relation between noon cortisol and loss of parents).

**Diurnal cortisol**

For the total group the basal cortisol curve showed a peak in the morning ($M = 9.49$, $SD = 7.05$, $n = 122$), with a decline to the noon ($M = 3.63$, $SD = 2.20$, $n = 120$) and afternoon levels ($M = 1.96$, $SD = 1.48$, $n = 128$). For the oldest age group, born between 1935 and 1937, the cortisol curve was less steep (morning: 0.91; noon: 0.45; afternoon: 0.26) than those of the other two age groups (morning: 0.84 and 0.90; noon: 0.49 and 0.51; afternoon: 0.15 and 0.10, respectively), also when we controlled for loss of parents and health status (see Figure 1). In a repeated measure analysis of covariance with morning, noon, and afternoon cortisol values as within-subject measures, loss of parents, depression, and physical health as covariates and age cohort as between-subject factors, the multivariate interaction between diurnal cortisol and age cohort, however, was not significant, $F(4, 204) = 2.22$, $p = 0.07$. The oldest age group tended to show a less steep decline from noon to afternoon cortisol level in comparison with the other two age groups (quadratic $F(2, 102) = 2.58$, $p = 0.08$). There were no main or interaction effects for gender. No significant differences were found between the values of the diurnal cortisol curve of the survivors with PTSD functional impairment and survivors without PTSD, $F(2, 103) = 0.28$, $p = 0.76$. Nor was there any interaction between PTSD and cortisol assessment, implying that there was no difference between the curves.
Table 1. Age

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>1935-1937</th>
<th>1938-1940</th>
<th>1941-1944</th>
<th>Total</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD) N</td>
<td>M (SD) N</td>
<td>M (SD) N</td>
<td>M (SD) N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>68.0 (0.79) 43</td>
<td>64.8 (0.88) 43</td>
<td>61.3 (1.10) 47</td>
<td>64.6 (2.94) 133</td>
<td>583.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Parents alive after war</td>
<td>1.37 (0.76) 43</td>
<td>1.60 (0.69) 43</td>
<td>1.74 (0.53) 47</td>
<td>1.58 (0.68) 133</td>
<td>3.59</td>
<td>0.03</td>
</tr>
<tr>
<td>Physical illness</td>
<td>2.72 (2.61) 43</td>
<td>1.95 (1.77) 43</td>
<td>1.96 (1.69) 47</td>
<td>2.20 (2.07) 133</td>
<td>2.01</td>
<td>0.14</td>
</tr>
<tr>
<td>Depression</td>
<td>2.40 (2.95) 43</td>
<td>2.42 (2.31) 43</td>
<td>2.32 (2.82) 47</td>
<td>2.38 (2.69) 133</td>
<td>0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>0.91 (.37) 37</td>
<td>0.84 (.28) 41</td>
<td>0.88 (.29) 44</td>
<td>0.87 (.31) 122</td>
<td>0.50</td>
<td>0.61</td>
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<td>Noon</td>
<td>0.48 (.25) 39</td>
<td>0.51 (.28) 39</td>
<td>0.49 (.21) 42</td>
<td>0.49 (.24) 120</td>
<td>0.09</td>
<td>0.91</td>
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<td>Afternoon</td>
<td>0.30 (.28) 41</td>
<td>0.18 (.29) 41</td>
<td>0.13 (.26) 46</td>
<td>0.20 (.28) 128</td>
<td>4.03</td>
<td>0.02</td>
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<td>20 min</td>
<td>-0.74 (.97) 34</td>
<td>-0.61 (.99) 34</td>
<td>-0.42 (1.21) 36</td>
<td>-0.59 (1.06) 104</td>
<td>0.79</td>
<td>0.46</td>
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<td>40 min</td>
<td>-0.53 (1.08) 36</td>
<td>-0.48 (1.13) 36</td>
<td>-0.77 (1.13) 36</td>
<td>-0.59 (1.06) 108</td>
<td>0.77</td>
<td>0.47</td>
</tr>
<tr>
<td>60 min</td>
<td>-0.74 (1.18) 33</td>
<td>-0.46 (1.06) 34</td>
<td>-0.64 (.91) 33</td>
<td>-0.61 (1.05) 100</td>
<td>0.60</td>
<td>0.55</td>
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<tr>
<td>Gender (Female)</td>
<td>22 (51%)</td>
<td>25 (58%)</td>
<td>34 (72%)</td>
<td>81 (61%)</td>
<td>$\chi^2 = 4.43$</td>
<td>0.11</td>
</tr>
<tr>
<td>Medication</td>
<td>35 (81%)</td>
<td>27 (64%)</td>
<td>32 (74%)</td>
<td>94 (73%)</td>
<td>$\chi^2 = 3.22$</td>
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<tr>
<td>PTSD</td>
<td>No PTSD</td>
<td>PTSD Functional Impairment</td>
<td>PTSD Not Reported</td>
<td>Total</td>
<td>$F$</td>
<td>$p$</td>
</tr>
<tr>
<td>----------------------</td>
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<td>$M$ (SD)</td>
<td>$N$</td>
<td>$M$ (SD)</td>
<td>$N$</td>
<td>$M$ (SD)</td>
<td>$N$</td>
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<tr>
<td>Age</td>
<td>65.0 (2.87)</td>
<td>32</td>
<td>65.0 (2.84)</td>
<td>38</td>
<td>64.2 (3.01)</td>
<td>63</td>
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<tr>
<td>Parents alive after war</td>
<td>1.63 (0.66)</td>
<td>32</td>
<td>1.37 (0.85)</td>
<td>38</td>
<td>1.68 (0.53)</td>
<td>63</td>
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<td>Physical illness</td>
<td>2.00 (1.32)</td>
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<td>3.08 (3.03)</td>
<td>38</td>
<td>1.78 (1.46)</td>
<td>63</td>
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<tr>
<td>Depression</td>
<td>1.84 (2.64)</td>
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<td>3.53 (3.17)</td>
<td>38</td>
<td>1.95 (2.19)</td>
<td>63</td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>18 (56%)</td>
<td>25 (66%)</td>
<td>38 (16%)</td>
<td>61%</td>
<td>81 (61%)</td>
<td>$X^2 = 0.68$</td>
</tr>
<tr>
<td>Medication</td>
<td>25 (86%)</td>
<td>28 (74%)</td>
<td>41 (67%)</td>
<td>73%</td>
<td>94 (73%)</td>
<td>$X^2 = 3.64$</td>
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Table 3. PTSD 2-way

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<tr>
<th>PTSD</th>
<th>PTSD Not Reported</th>
<th>PTSD Functional Impairment</th>
<th>Total</th>
<th>F</th>
<th>P</th>
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<td>( M (SD) N )</td>
<td>( M (SD) N )</td>
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<tr>
<td>Age</td>
<td>64.5 (2.97) 95</td>
<td>65.0 (2.84) 38</td>
<td>64.6 (2.94) 133</td>
<td>1.04</td>
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<td>Parents alive after war</td>
<td>1.66 (0.58) 95</td>
<td>1.37 (0.85) 38</td>
<td>1.58 (0.68) 133</td>
<td>5.32</td>
<td>0.02</td>
</tr>
<tr>
<td>Physical illness</td>
<td>1.85 (1.41) 95</td>
<td>3.08 (3.03) 38</td>
<td>2.20 (2.07) 133</td>
<td>10.15</td>
<td>&lt;0.01</td>
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<td>Depression</td>
<td>1.92 (2.34) 95</td>
<td>3.53 (3.17) 38</td>
<td>2.38 (2.69) 133</td>
<td>10.42</td>
<td>&lt;0.01</td>
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<td>Gender Female</td>
<td>56 (59%)</td>
<td>25 (66%)</td>
<td>81 (61%)</td>
<td>0.53</td>
<td>0.47</td>
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<tr>
<td>Medication</td>
<td>66 (73%)</td>
<td>28 (74%)</td>
<td>94 (73%)</td>
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</tr>
<tr>
<td>Morning</td>
<td>0.87 (0.30) 86</td>
<td>0.89 (0.34) 36</td>
<td>0.87 (0.31) 122</td>
<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td>Noon</td>
<td>0.47 (0.26) 84</td>
<td>0.53 (0.19) 36</td>
<td>0.49 (0.24) 120</td>
<td>1.59</td>
<td>0.21</td>
</tr>
<tr>
<td>Afternoon</td>
<td>0.18 (0.29) 92</td>
<td>0.25 (0.27) 36</td>
<td>0.20 (0.28) 128</td>
<td>1.78</td>
<td>0.18</td>
</tr>
<tr>
<td>Reactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 min</td>
<td>-0.60 (1.06) 73</td>
<td>-0.56 (1.07) 31</td>
<td>-0.59 (1.06) 104</td>
<td>0.04</td>
<td>0.85</td>
</tr>
<tr>
<td>40 min</td>
<td>-0.57 (1.02) 74</td>
<td>-0.65 (1.16) 34</td>
<td>-0.59 (1.06) 108</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td>60 min</td>
<td>-0.58 (1.00) 67</td>
<td>-0.69 (1.17) 33</td>
<td>-0.61 (1.05) 100</td>
<td>0.24</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Stress reactivity

Reactivity at 20 minutes after onset of the test session was established by subtracting the standardized residual of the regression of the basal on the cortisol level from the time equivalent basal cortisol level after 20 min, thereby controlling for differences in baseline cortisol level. We tested for difference in stress reactivity among the three age groups with gender as a second factor and loss of parents, depression and physical health as covariates. The interaction between age group and gender was significant, \(F(2, 95) = 3.13, p = 0.048\), see Figure 2. The males in the youngest age group showed the strongest reactivity. No significant main or interaction effects were found at 40 and 60 minutes after the test session began, although differences were in the same direction.

In an analysis of covariance between the two PTSD groups with gender as a second factor and loss of parents, depression and physical health as covariates, the interaction between PTSD functional impairment and gender was significant for reactivity at 20 minutes from the beginning of the session, \(F(1, 97) = 3.97, p = 0.049\), see Figure 3. Males with functional PTSD impairment showed the strongest reactivity. No significant main or interaction effects were found at 40 and 60 minutes after the test session began, although, again, differences were in the same direction.
Figure 3 Cortisol reactivity for respondents with and without functional PTSD impairment

Note
Reactivity controlled for physical health, depression, and loss of parents during war.

Discussion

The current study provides evidence that the youngest survivors of the Nazi persecution bear late life effects of traumatic stress during early childhood. In our study age, parental loss during the Holocaust, current depression and physical illness were not associated with differences in basal diurnal cortisol levels. However, we noticed neuroendocrine effects in stress reactivity through elevated cortisol levels in the youngest male age group, and in male respondents suffering from PTSD-related functional impairment. Furthermore, we found a prominent association of depression with parental loss during the war. Child survivors who had lost both parents during the Holocaust were significantly more depressed than survivors who lost one parent, or did not suffer parental loss. Finally, survivors affected by PTSD-functional impairment also suffered significantly more often from physical illnesses and depression.

Limitations

One important limitation of our study concerns the absence of a control group of the same age cohort, not persecuted by the Nazis, or in other ways
affected by the consequences of the Second World War. Selecting a matched comparison group was too difficult to accomplish during the time frame available for the current study. We therefore focused on individual differences among child Holocaust survivors, with special emphasis on time of their birth. Another limitation is the incomplete data on cortisol as we started data collection halfway through our study; nevertheless, this rendered a substantially large group of (unselected) participants. We invested great efforts in oral and written instructions for taking saliva samples, and encouraged our subjects to keep contact for questions and prompts. Despite this, subject compliance remained a problem in home collection studies (Yehuda et al., 2003). Failure to adhere to instructions may possibly have affected the reported findings, although there is no reason to suspect a systematic bias. Moreover, the effects of a stressor on cortisol reactivity found in our study were based on saliva samples that were taken in the presence of research assistants during a standardized procedure.

Loss and Depression

We are not sure why the oldest group of survivors in our sample more often suffered the loss of both parents than the two younger groups. One explanation could be that the older children, though still toddlers in many cases, were more often handed over to the care of strangers to keep them hidden from persecution. Parents probably more often intuitively kept their youngest children, when they were still babies, in their own charge, as they tried to escape deportation.

An association between the adversity of early parental loss and depression in later life has been observed already by Keilson (1992). In his longitudinal study on Dutch-born orphaned child survivors he draws attention to the prevalence of depressive mood in these survivors in adult life. On the other hand, Robinson, Rapaport-Bar-Sever & Rapaport (1997), examined different aspects of the psychosocial effects of the Holocaust in 103 child survivors and found no differences in depressive complaints between survivors who lost both parents and those who lost none or one parent. In non-Holocaust-related studies, Agid et al. (1999) found that parental loss during childhood, especially before the age of 9, contributed significantly to developing major depression in adult life, with loss due to permanent separation being even more devastating than loss due to death.

Diurnal Cortisol

Our data show little evidence for associations between diurnal cortisol and coping with the hardships of traumatic early life experiences, such as parental loss as a result of the war. Since to our knowledge no other studies of these measures involving child Holocaust survivors have been published, we compared our findings with non-Holocaust-related studies. Nicolson (2004) found higher basal
cortisol levels in healthy adult men who lost a parent during childhood compared to controls who did not suffer parental loss. Luecken (2000) mentioned subsequent quality of care provided by the remaining parent or other caregiver, or other adversities during childhood as additional risk factors for neuro-endocrine effects related to parental loss. From these findings we infer that appropriate care after parental loss may be a reason for the absence of deviating diurnal cortisol patterns in our sample (Van der Hal-Van Raalte, Van IJzendoorn & Bakermans-Kranenburg - in press).

Elevated levels of basal cortisol have quite consistently been associated with mood disorders (Heim, Plotsky & Nemeroff, 2004; Plotsky, Owens & Nemeroff, 1998). In our study we were unable to find such a connection, nor were there any associations between decreased levels of diurnal cortisol and physical impairment or PTSD. Heim, Ehlert & Hellhammer (2000) reported decreased cortisol levels for healthy individuals living under stressful conditions, and for patients suffering from stress-related disorders, such as CFS, fibromyalgia, rheumatoid arthritis and asthma. In our study we assessed PTSD functional impairment, and the associations we found with physical illness and depression are consistent with findings in non-Holocaust-related studies of military veterans and civilian populations (Deykin, Keane, Kaloupec, Fincke, Siegfried, et al., 2001; Dobie et al., 2004; Ford et al., 2001; Zatzcick et al., 1997). Moreover, our findings are consistent with other studies reflecting on clinical observations of heightened psycho-social vulnerability of child survivors, even when they seem outwardly well-adapted (Cohen, Brom, et al., 2001; Dasberg, Bartura & Amit, 2001).

The tendency in our results for the oldest age group to show a less steep decline of cortisol level over the day confirmed findings of Ferrari et al. (2001). They noticed that with physiological and pathological aging a relative increase of cortisol serum levels in the evening and at night-time is responsible for a flattened cortisol circadian profile. In an earlier study, Yehuda et al. (1995) found lower mean 24-hour urinary cortisol excretion in Holocaust survivors with PTSD than in Holocaust survivors without PTSD. In that study, cortisol levels were significantly related to the severity of PTSD, due to a substantial association between cortisol levels and scores on the PTSD avoidance subscale. In the current study, we did not find similar associations with the PTSD functional impairment scale or with the PTSD symptoms scales of the PDS (Foa et al., 1997).

Cortisol Reactivity

Although the literature concerning cortisol reactivity to a stressor is not entirely consistent, many studies report stronger cortisol reactivity with aged male participants (Kudielka et al., 2004; Kudielka et al., 1998; Traustadottir, Bosch & Matt, 2003; Wolf et al., 2001). Our finding of higher cortisol reactivity of the males
in our sample was thus not unexpected. An interaction effect for cortisol reactivity between gender and PTSD was also found in Hawk, Dougall, Ursano & Baum (2000). In their study they found elevated urinary cortisol levels among PTSD-symptomatic men, but not women, one month after a motor vehicle accident. In many areas related to physical and psychological growth, males have been found to be developmentally more vulnerable than females. (Nagy, Loveland, Orvos & Molner, 2001).

A most interesting outcome concerns the significant interaction between age group and gender, by which males in the youngest age group showed the strongest reactivity. Although the age differences between the groups are not large, it bears historical significance: The survivors in the youngest group were all born after the outbreak of the war and after the Nazi persecution had started. We infer from our results that the youngest survivors in our study show neuro-endocrine reactions while under stress that are significantly different from the reactions of survivors born before the onset of persecution.

In view of the important role of sensitive and responsive parenting in buffering reactivity of the HPA system to potentially stressful events (Gunnar, 1998; Gunnar et al., 1996; Larson et al., 1998), it stands to reason that the older child survivors in our sample, born before the onset of the persecution, could more often rely on parental care not yet compromised by the stresses of coping with moment-to-moment survival. They were still able to enjoy their parents’ ‘good enough’ support for regulating and buffering normal infantile internal and environmental stresses and anxieties, at least in the first years of their lives (Siegel, 1999; Stern, 1985). Born after the onset of Nazi persecution, the youngest participants in our study were, due to the circumstances, more often deprived of unconditional care and attention by their parents or substitute parents. Furthermore, the youngest survivors may also have suffered more from pre- and perinatal stresses affecting HPA-axis functioning already before birth (De Weerth et al., 2003). Our findings leave room for prenatal programming of the neuro-endocrine system (Bertram & Hanson, 2002), in infants of mothers who were stressed by the extreme circumstances of the Holocaust. Thus, our findings support the concept of an early onset of differential neuroendocrine pathways to stress-regulating strategies of children born in the midst of war and genocide.