Effects of interpersonal violence-related post-traumatic stress disorder (PTSD) on mother and child diurnal cortisol rhythm and cortisol reactivity to a laboratory stressor involving separation

Maria I. Cordero¹²*, Dominik A. Moser², Aurelia Manini², Francesca Suardi², Ana Sancho Rossignol², Raffaella Torrisi², Michel F. Rossier³, François Ansermet²⁴, Alexandre G. Dayer²⁴, Sandra Rusconi Serpa² and Daniel S. Schechter²⁴⁵.

¹ Faculty of Health, Psychology and Social Care, Manchester Metropolitan University, Manchester, UK

² Research Unit, Child and Adolescent Psychiatry Service, University of Geneva Hospitals, Geneva, Switzerland

³ Clinical Chemistry and Toxicology Service, Hôpital du Valais, Sion, Switzerland

⁴ Department of Psychiatry, Faculty of Medicine, University of Geneva, Geneva, Switzerland

⁵ Division of Developmental Neuroscience, Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY, USA

* Corresponding author:

Dr. Maria Isabel Cordero
Faculty of Health, Psychology and Social Care
Manchester Metropolitan University
Brooks Building
53 Bonsall Street
Manchester
M15 6GX
United Kingdom

Tel.: +44 (0)161 247 2588
m.cordero@mmu.ac.uk

Count:
Abstract: 245
Total word-count: 6646
Figures: 4
Tables: 3
Total references: 73
Abstract

Women who have experienced interpersonal violence (IPV) are at a higher risk to develop posttraumatic stress disorder (PTSD), with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and impaired social behavior. Previously, we had reported impaired maternal sensitivity and increased difficulty in identifying emotions (i.e. alexithymia) among IPV-PTSD mothers. One of the aims of the present study was to examine maternal IPV-PTSD salivary cortisol levels diurnally and reactive to their child’s distress in relation to maternal alexithymia. Given that mother-child interaction during infancy and early childhood has important long-term consequences on the stress response system, toddlers’ cortisol levels were assessed during the day and in response to a laboratory stressor. Mothers collected their own and their 12-48 month-old toddlers’ salivary samples at home three times: 30 min after waking up, between 2-3 pm and at bedtime. Moreover, mother-child dyads participated in a 120-min laboratory session, consisting of 3 phases: baseline, stress situation (involving mother-child separation and exposure to novelty) and a 60-min regulation phase. Compared to non-PTSD controls, IPV-PTSD mothers -but not their toddlers-, had lower morning cortisol and higher bedtime cortisol levels. As expected, IPV-PTSD mothers and their children showed blunted cortisol reactivity to the laboratory stressor. Maternal cortisol levels were negatively correlated to difficulty in identifying emotions. Our data highlights PTSD-IPV-related alterations in the HPA system and its relevance to maternal behavior. Toddlers of IPV-PTSD mothers also showed an altered pattern of cortisol reactivity to stress that potentially may predispose them to later psychological disorders.

Keywords max 10: interpersonal violence; PTSD; toddlers; cortisol; alexythimia; early childhood; risk; intergenerational; glucorticoids; HPA-axis
Introduction

Exposure to interpersonal violence (IPV), particularly when perpetrated by an intimate partner, has been related to adverse physical and mental health problems (Ellsberg et al., 2008; Lagdon et al., 2014). Women who have experienced IPV have a high probability of suffering from psychiatric disorders, such as posttraumatic stress disorder (PTSD), which in turn can affect parenting (Satyanarayana et al., 2015; Schechter et al., 2010; Trevillion et al., 2012).

The diagnosis of PTSD requires that the individual presents the following symptom clusters: re-experiencing or intrusion symptoms, effortful avoidance, hyperarousal and negative cognitions and mood including emotional numbing (A.P.A., 2013). Detrimental effects of IPV-PTSD such as emotional numbing have important consequences on the relationships with other family members. In fact, it has been pointed out that an important pathway by which intimate partner violence can affect child development is by affecting maternal mental health and behavior and thus the mother-child relationship during the first years of life (Maddoux et al., 2016; Symes et al., 2016). For example, IPV-PTSD mothers show impaired reading of the emotion of the child having less empathic responses (Lyons-Ruth and Block, 1996; Schechter et al., 2010), and thus less sensitive maternal behavior [e.g., awareness of child signals, accurate interpretation and prompt and appropriate response, (Schechter et al., 2015b)]. The aforementioned studies converge in identifying sensitive maternal behavior as a key factor influencing a healthy child development. Understanding the neurobiological alterations underlying maternal psychopathology that is related to IPV is not only crucial in the treatment of parents but also a priority in order to prevent the deleterious effects on their children.
In response to stress, the hypothalamic-pituitary-adrenal (HPA) axis is activated, and as a consequence, the synthesis and release of glucocorticoids (i.e. cortisol in humans) increases in order to mobilize energy to help the organism to confront the stressor (de Kloet et al., 2005). Glucocorticoids released into the blood system enter the brain, and through a negative feedback loop, promote the inhibition of the HPA-axis (de Kloet et al., 2005). Posttraumatic stress disorder following repetitive traumatic experiences, such as IPV or combat, has been associated with a reduction of cortisol levels, both in terms of circadian rhythm and in response to stress (Daskalakis et al., 2013). Relevant to the present study, low maternal cortisol levels have been associated with a lack of sensitivity to distressed infant cues (Crockett et al., 2013). In addition, in a cohort of mothers with IPV-PTSD, one previous study (Schechter et al., 2004) found that maternal IPV-PTSD severity and atypical maternal behavior were associated with a low salivary cortisol level at baseline and blunted cortisol reactivity in response to mother-child separation. In a similar vein, authors of the present paper recently showed that lower methylation of the glucocorticoid receptor gene \textit{NR3C1} was associated with i) higher maternal PTSD severity and ii) lower maternal medial prefrontal cortex activity, the latter being a key brain area for emotion processing in response to video-stimuli of mother-child separation versus play (Schechter et al., 2015a). Low methylation of the glucocorticoid receptor gene \textit{NR3C1} is considered as a marker for HPA-axis functioning with less methylation likely to be associated with a greater production of glucocorticoid receptors, and thus less circulating cortisol. Altogether, findings support the hypothesis that parenting impairment in response to child stress, as observed among mothers with IPV-PTSD, might be related to the neurobiological alterations in brain activity mediated by low cortisol levels. In spite of previous findings, it is still not known how cortisol response at the time of child distress differs between IPV-PTSD mothers and a normative sample of control mothers (healthy
control, HC), and whether maternal cortisol response is related to child’s distress behavior.

Exposure to intense stress during early life (such as for example, war, natural catastrophes, or parental IPV) can have long-term effects on HPA-axis functioning, affect the development of critical brain areas, and increase the risk to develop later psychopathology (Dannlowski et al., 2012; Essex et al., 2011). Given that maternal PTSD has been identified as a major contributor to transgenerational transmission of PTSD and offspring’s low cortisol levels (Bader et al., 2014; Lehrner et al., 2014; Yehuda et al., 2008; Yehuda and Bierer, 2008), toddlers exposed to parental violence who have mothers with PTSD could have an increased risk to psychopathology as related to HPA-axis alterations. Therefore, it is of great interest to study the effects of IPV and maternal PTSD on young children’s HPA-axis functioning.

Based on the literature reviewed above, we expected that IPV-PTSD mothers and their toddlers would have a low diurnal cortisol release pattern, and blunted cortisol reactivity to stress. Furthermore, given that we found impaired maternal sensitivity and increased difficulty in identifying emotions in IPV-PTSD women from this same cohort (Schechter et al., 2015b), and lower activity in key brain areas for emotion processing while watching a video showing child separation distress versus play (Schechter et al., 2015a), we expected i) that maternal cortisol reactivity to child distress would negatively correlate with difficulty in identifying emotions, and ii) that IPV-PTSD mothers would be impaired in rating distress levels of their toddlers during the laboratory stressor.

**Material and Methods**

This study was done during the first phase of an ongoing longitudinal study of the effects of interpersonal violence and related maternal psychopathology on developmental
psychopathology of the child. It was approved by the Institutional Review Board of the University of Geneva Hospitals and Faculty of Medicine, in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). The study involved maternal and child psychiatric assessment, mother-child behavioral assessment during which we assessed mother-child salivary cortisol response to routine stressors (separation-reunion, novelty, frustration, as described in more detail below), and fMRI scanning of maternal neural activation in response to silent video clips of children and adults. Some of those findings—none of which included direct cortisol measurements—have been published and data are not included in the current article (Moser et al., 2015a; Moser et al., 2015b; Moser et al., 2015c; Schechter et al., 2015a; Schechter et al., 2015b).

Participants

Recruitment: Participants were recruited via flyers posted in the local community. Participants gave informed written consent before participation in the study. Only biological mothers with toddlers between 12-48 months-old were asked to participate. Interested subject-mothers were excluded during screening if they were pregnant or lactating, actively substance or alcohol abusing, psychotic, taking medication or having a medical illness with known effect on HPA-axis functioning [e.g., oral glucocorticoid medication, (Granger et al., 2009)]. Mothers and children were excluded if they suffered from a physical or mental handicap that would preclude full participation in the study tasks. After each visit, mothers received 50 Swiss francs along with a small book or toy for their child.

Diagnosis: During an initial videotaped interview that took place at least one week before the mother-child behavioral observation, participant-mothers were interviewed by experienced clinicians using the Clinician Administered PTSD Scale [CAPS, (Blake et
al., 1995)] to assess PTSD with confirmation of the severity of their current PTSD symptoms via the Posttraumatic Symptom Checklist - short version [PCL-S, (Weathers et al., 2001)]. Following our previous work (Schechter et al., 2010), and in order to ensure a clear diagnoses of PTSD versus subjects who did not have clinically significant symptoms of PTSD at a subthreshold level, we selected CAPS and PCL-S score thresholds based on previous literature (Griffin et al., 2004; Ventureyra et al., 2002). Participants were diagnosed as having IPV-PTSD if CAPS score $\geq 55$ and PCL-S score $\geq 40$; participants with CAPS score $< 30$, and PCL-S score $< 25$ were considered as normative control sample (HC). If one of the scores was in between those values, participants were excluded from the analyses in the present study. In the IPV-PTSD group only participants who met full DSM-V criteria for PTSD (A.P.A., 2013) that was related to IPV were included. Participants for whom responses to the psychological tests were found not to be reliable were excluded from the analyses. For the current study, 45 mothers (HC, $n=18$; IPV-PTSD, $n=27$) and their young children (12-42 months of age) were included in the analyses.

**Measures**

**Socio-demographic variables**

During the screening session we conducted an interview with the mothers using the Geneva Socio-demographic Questionnaire (Sancho-Rossignol et al., 2010) which was adapted from the Structured Clinical Interview for the DSM-IV (First et al., 1995) and developed for the present study in order to obtain a detailed overview of the parents’ ethnic background, characteristics and history of the mother-partner relationships, and exposure to stressful life-events (i.e. interpersonal violence, past substance abuse, economic difficulties, immigration, and physical and mental health problems and interventions, and child protective and judicial services involvement). The family socio-
economic status (SES) was calculated using the Largo Index (Largo et al., 1989), a well-validated SES index that takes into account both parental educational attainment and occupational status.

**Alexithymia, subscale difficulty identifying feelings**

Alexithymia [difficulty in identifying, expressing and describing emotions (Sifneos, 1973)] was assessed using the well-validated French language version of the Toronto Alexithymia Scale (TAS-20) which consists of 20 items (Loas et al., 1997). The TAS-20 and its component subscales demonstrate good internal consistency (Cronbach’s alpha .81) and test–retest reliability (.77). Its 3 component subscales are: difficulty describing feelings; difficulty identifying feelings; externally-oriented thinking (Bagby et al., 1994). For this study, we specifically used the “Difficulty Identifying Feelings” subscale to test our *a priori* hypothesis that maternal cortisol reactivity to child distress would negatively correlate with difficulty in identifying emotions.

**Laboratory session**

Mothers were given an appointment to bring their child to the lab, and asked to cancel if either of them had any acute medical condition that could affect HPA-axis functioning (i.e. fever, fatigue). Mothers and children were told not to eat or drink, or brush their teeth at least 30 min prior to the appointment, and mothers were instructed not to smoke or use lipstick.

The mother-child behavioral observations and saliva sampling were performed during a laboratory session that consisted of a 30-min mother-child interaction procedure known as the “Modified Crowell Procedure” (Zeanah et al., 2000), which consisted of free and structured play, separation-reunion, clean-up and exposure to novelty as described in detail below. Upon arrival at the lab (appointments were scheduled between
the mother and child were greeted by a clinician and an assistant technician. During the following 10-min the mothers were asked to complete a questionnaire collecting information about the day, time mother and child awakened, and when they had last eaten and drank. They were asked to describe activities and events that occurred during the day, mothers’ recent menstrual cycle, waking up time, previous night’s hours of sleep, their state of physical and mental health, and to list any medications that they had recently taken. All of these variables were carefully examined in order to ensure participants could be included in the analysis. Afterwards, the mothers were given instructions about how the session would proceed (see, Fig.1). Briefly, the session began with 8 min of mother-child free play, followed by 3-min separation (mother left the room leaving the child on their own). After the mother returned and resumed playing with the child for 2-min, the clinician entered the room and asked them to clean-up (2-min). Following this, mother and child played with a puzzle for 4 min, followed by a 3-min separation. Two min after reunion, an assistant wearing a clown’s clothes and mask entered the room and during 3-min tried to interact in a non-intrusive, gentle way with the child. Once the assistant left, the clinician returned with a mechanical dinosaur that played in front of the toddler (the dinosaur moved and growled) and tried to engage the child to play with it. The mother-child interaction procedure was followed by administration of measures focusing on the child's life events and behavior, which are not included in analyses in this study.

Behavioral scoring of toddlers’ distress display

Toddlers’ distress behavior was scored during the laboratory stressors. During mother-child separation episodes, the following toddlers’ behaviors were scored (scale 0-3; 0=none, 3=strong display): resistance of the child to the mother leaving the room, crying, vocalizations calling the mother and approaching the door. During clown and
dinosaur episodes, the behaviors scored were the following: crying, avoiding the stimuli, attempt to be held by the mother (scale 0-3; 0=none, 3=strong display); smiling and playing with the stimuli (scale 0=strong display, 3=none). Total child distress for each of these segments was calculated by adding up all the scores during the stressor portion of the Modified Crowell Procedure and standardized as percent for comparison.

During training coders were required to reach a minimum training reliability intraclass correlation coefficient = 0.8.

Cortisol stress-response to a laboratory stressor involving separation

For purposes of salivary cortisol measurement, the “laboratory stressor” consisted of two 3-min separations from the mother with 8 min interval between them, and exposure to two novel stimuli. Novel stimuli consisted of a two, 3-min long segments during which the child and mother were i) approached by a stranger dressed as a clown who honked a squeeze-horn and after the stranger left, ii) exposure to a remote-controlled dinosaur robot that was positioned to walk towards the child and make a roaring noise. Four salivary samples for the assessment of cortisol were collected from the mother and the child: baseline (before the procedure begin), immediately after the end of the stress situation (T0), and at 30 (T30) and 60 (T60) min after the end of the experimental condition and during the recovery phase (Fig.1). Each time saliva samples were collected, the mothers were asked to estimate their own and their child’s anxiety levels on a Lickert scale of 0-5 (0=almost sleeping, 5=very anxious). Sampling of cortisol was obtained using the Salivette® system (Salivette®, Sarstedt Inc., Rommelsdorf, Germany); www.sarstedt.com) in which a dry cotton swab is placed in the mouth for 2-3 seconds to passively absorb saliva. To avoid potential choking hazard, cotton swabs used for toddlers were tied with dental flushing robe and the mothers collecting the samples held the robe all the time. Mothers were instructed to moisten the end of the cotton by placing it briefly
in the toddler’s mouth, and then dipping it in a child’s bowl containing sugar to promote salivation. Similar procedures to collect saliva collection from young children have been used, rigorously tested and carefully examined for possible effect on assay measurements and found no significant effect on cortisol values (Gordon et al., 2005; Talge et al., 2005). Given the difficulty of collecting saliva samples from the toddlers, especially 12-24 months-old, Salivette® cottons were inserted inside a disposable perforated pacifier; and the mothers were instructed how to collect saliva samples and asked to collect them from their toddlers in a comfortable, stress-free way. If this was not possible and the toddlers showed distress symptoms, the samples were not collected. With the aim of encouraging the toddlers to collaborate, a cartoon sticker was given to the toddlers after each successful saliva collection. At the end of the session, samples were frozen at -20 °C until cortisol assay.

**Morning, afternoon and night cortisol levels**

At the end of the aforementioned laboratory session, mothers were provided with a pack containing Salivette® swabs, disposable perforated pacifier, written instructions on saliva collection, self-report forms and asked to collect saliva from themselves and their toddlers 30 min after waking, in the afternoon (between 2-3pm), and prior to bedtime on what the mother considered to be the least stressful day of the week. Participants were instructed not to eat or drink, smoke or brush teeth for at least 30 min prior to saliva collection. They were told to store the samples in the freezer immediately after collection and bring them to the laboratory on the next visit (within the following month).

Participants were asked to record the date and time of collection in addition to their estimation of their own and their child’s anxiety-level at the time of collection, along with notation of their time of awakening, child-nap schedule, use of medication, and state of health. They were also instructed to provide a brief description of the events on the
collection day. All of this information was collected in order to assess adherence to sampling instructions and possible confounding variables.

**Hormone Assays**

Salivary cortisol assays capture the free unbound fraction of the total hormone that is biologically active (Bober et al., 1988). Saliva samples were frozen at -20 °C until cortisol assay. Upon thawing, samples were centrifuged (2 min at 1000 rpm), the swab was discarded and 100 µl saliva aliquots were collected. Cortisol concentration was measured directly (without extraction) on a cobas e-601 analyzer (Roche) by an electrochemiluminescent immunoassay (ECLIA). For this purpose, a biotinylated polyclonal sheep anti-cortisol antibody was used to bind, in a competition mode, both endogenous salivary cortisol and a synthetic cortisol derivative labeled with ruthenium. After incubation, bound cortisol was separated from the free fraction by addition of streptavidin-coated beads and the signal measured according to the Roche Elecsys protocol. The analytical detection limit of the assay in saliva was 0.5 nmol/l and inter-assay imprecision at 8.0 nmol/l was 11.5% (coefficient of variation, CV), while intra-assay CV was < 6.0 % at the same concentration. The whole analytical procedure was accredited according to the international ISO-15189 laboratory norms.

**Data analysis**

Unless specified otherwise, results are expressed as mean±SEM. Statistical analyses were performed using SPSS Version 21.0. We used Student’s *t*-test and Pearson’s $\chi^2$ to compare demographical data between groups (HC vs. IPV-PTSD). Cortisol values were first examined for outliers and for skew. Effect of IPV-PTSD on cortisol levels during the laboratory session and diurnal release were analyzed using ANOVAs with repeated measurements. In case of a significant group by condition
interaction effect, post-hoc $t$ tests were performed. We used Student $t$- test for between-subject comparisons of cortisol levels at different time points and paired $t$-test for within-subject comparisons. Mann–Whitney-$U$ tests was used for between-subject comparisons of anxiety levels and behavioral distress at different time points, and Wilcoxon–Signed–Rank tests for within-subject comparisons. Partial $\eta^2$ and Cohen’s $d$ are reported as measures of effect sizes for ANOVAs and pair-wise comparisons, respectively. Spearman’s $r_s$ was used to examine correlations between anxiety levels and behavioral distress, and between alexithymia subscale difficulty identifying feelings and cortisol levels.

**Results**

**Sociodemographic information**

Mothers and fathers of the child-participants were from a variety of ethnic backgrounds (Table 1). Pearson $\chi^2$ showed significant differences in mothers’ ethnic background, 1/3 of IPV-PTSD mothers were non-caucasian (African and Hispanic) while all HC mothers were caucasian [$\chi^2(2,n=45)=7.5, p<0.03$]. No significant differences were found for fathers’ [$\chi^2(2,n=45)=2.52, p>0.19$] or children’s [$\chi^2(3,n=45)=2.6, p>0.27$] ethnic background.

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>African</th>
<th>Hispanic</th>
<th>Mixed Toddlers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M F T</td>
<td>M F T</td>
<td>M F T</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>18 17 9</td>
<td>0 1 0</td>
<td>0 0 0</td>
<td>1</td>
</tr>
<tr>
<td>IPV-PTSD</td>
<td>18 21 10</td>
<td>2 4 2</td>
<td>7 2 0</td>
<td>5</td>
</tr>
</tbody>
</table>

*Table 1. Ethnic background distribution of mothers (M), fathers (F) and toddlers (T) within the groups.*

No differences between groups were found in terms of mothers’ and fathers’ age (mothers: HC=35±1.4, IPV-PTSD=34±1.3, $t(43)=0.5, p>0.62$; fathers: HC=36±1.3, IPV-
PTSD=38.7±1.6, \( t(43)=-1.1, p>0.26 \), mothers’ civil state [married (\( n=29 \)), not married living with a couple (\( n=4 \)), single (\( n=7 \)), separated (\( n=3 \)), divorced (\( n=2 \)); \( \chi^2(4, n=45)=4.7, p>0.31 \)]. The number of children in the family was not different between the groups [one (\( n=17 \)), two (\( n=17 \)), three (\( n=8 \)), four (\( n=2 \)); \( \chi^2(3, n=45)=3.36, p>0.49 \)]. However, control mothers were of higher SES than IPV-PTSD mothers, (SES range:2-10, lower scores depict higher SES; HC=4.4±.43, IPV-PTSD=6.3±0.4; \( \chi^2(8, n=45)=20.79, p<0.01 \)).

During childhood maternal experience of violence, sexual abuse and exposure to domestic violence were significantly higher among IPV-PTSD than in HC mothers (Table 2). Adult physical assault but not sexual assault was also higher among IPV-PTSD mothers compared to HC mothers (Table 2). Furthermore, IPV-PTSD mothers experienced significantly more violent events since childhood than non-PTSD controls (HC, mean rank=16.08, IPV-PTSD, mean rank=27.61, Mann-Whitney-test, \( n=45, U=367, Z=3.096, p<0.002 \)).

<table>
<thead>
<tr>
<th>Type and timing of maternal IPV exposure</th>
<th>HC</th>
<th>IPV-PTSD</th>
<th>Statistical analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal experience of violence during childhood</td>
<td>0%</td>
<td>30%</td>
<td>( \chi^2(1, n=45)=6.49, p&lt;.05 )</td>
</tr>
<tr>
<td>Maternal experience of sexual abuse during childhood</td>
<td>0%</td>
<td>19%</td>
<td>( \chi^2(1, n=45)=3.91, p&lt;.05 )</td>
</tr>
<tr>
<td>Maternal experience of exposure to domestic violence during childhood</td>
<td>17%</td>
<td>52%</td>
<td>( \chi^2(1, n=45)=6.49, p&lt;.01 )</td>
</tr>
<tr>
<td>Adult physical assault</td>
<td>17%</td>
<td>48%</td>
<td>( \chi^2(1, n=45)=4.67, p&lt;.05 )</td>
</tr>
<tr>
<td>Adult sexual assault</td>
<td>6%</td>
<td>30%</td>
<td>( \chi^2(1, n=45)=2.44, p&gt;.2 )</td>
</tr>
</tbody>
</table>

Table 2. Type and timing of exposure to interpersonal violence of HC and IPV-PTSD mothers.

Regarding the number of children included in the cortisol analyses, both groups had a similar distribution of child age [Table 3: \( \chi^2(2, n=27)=0.034, p>0.98 \) and gender [\( \chi^2(1, n=27)=0.91, p>0.33 \).]
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HC</td>
<td>Male</td>
</tr>
<tr>
<td>IPV-PTSD</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3. Distribution of children’s gender and age within the groups.

**Cortisol reactivity to the laboratory stressor**

Group differences (HC vs IPV-PTSD) on salivary cortisol stress reactivity during the course of the laboratory session was assessed by collection of salivary samples before the procedure began (baseline), immediately after it finished (T0), and 30 min and 60 min afterward it ended, during which time, participants completed non-stressful tasks (T30, T60).

ANOVA repeated measures of mothers’ cortisol levels during the laboratory stressor showed a significant effect for time \((F_{3,129}=29.03, p<0.001, \eta^2_p=0.41)\) and groupxtime interaction \((F_{3,129}=3.63, p<0.015, \eta^2_p=0.08)\), but no significant effect for group \((F_{1,43}=2.7, p>0.2, \eta^2_p=0.06)\). Post-hoc analyses showed significant differences in mothers’ cortisol levels immediately after stress (T0), with IPV-PTSD mothers showing lower levels compared to HC mothers \([t(43)=2.2, p<0.04, d=-0.67]\). Yet no differences were found at the baseline \([t(43)=1.3, p>0.2, d=-0.39]\) or during recovery \([T30 t(43)=1.73, p=0.091, d=-0.53; T60; t(43)= -0.6, p>0.6, d =-0.18]\). Interestingly, in the IPV-PTSD group, but not in the control group, cortisol levels immediately after the stressor were significantly lower than the baseline cortisol level \([HC: t(17)=0.36, p>0.72, d=0.05; IPV-PTSD: t(26)=5.32, p<0.001,d=0.45]\). In both groups, cortisol levels continue to decrease significantly at 30 min and 60 min after stress, compared to baseline and immediately after stress \([HC: ts(17)=3.65-5.38, ps<0.002, ds=0.41-0.98; IPV-PTSD: ts(26)=2.4-5.13, ps<0.03, ds=0.25-0.72]\).
The collection of the children’s saliva samples was at times difficult (i.e. some toddlers regardless of group membership did not cooperate; and, not infrequently, the quantity of saliva at a given time-point was insufficient). For this reason, child cortisol data was analyzed in two ways. First, we performed an ANOVA repeated measures of child cortisol levels during the laboratory stressor. ANOVA repeated measures showed a significant effect for groupxtime interaction (Fig.2B; $F_{3,75}=2.9$, $p<0.05$, $\eta^2_p=0.1$; HC:$n=10$, IPV-PTSD:$n=17$), group ($F_{1,25}=0.28$, $p>0.6$, $\eta^2_p=0.01$), and time ($F_{3,75}=1.9$, $p>0.17$, $\eta^2_p=0.07$) were not significant. Note that in order to perform these analyses, only data from those children who provided enough saliva to perform cortisol analyses in each one of the four samples taken during the laboratory stressor were included. With the aim of including the maximum number of toddlers in the analyses, we performed Student $t$-test analyses of cortisol samples at each time point. Compared to control mothers’ children, IPV-PTSD mothers’ children had significant lower levels of cortisol 30 min after the end of the last stressor [T30: HC=9.8±1.34, $n=17$; PTSD=6.25±0.7, $n=22$; $t(37)=2.49, p<0.02, d=0.76$]. No significant differences were found between the groups at baseline [$t(29)=-0.40$, $p>0.69$, $d=-0.16$], T0 [$t(31)=0.38$, $p>0.7$, $d=0.13$] or T60 [$t(36)=0.65$, $p>0.52$, $d=0.21$].

Cortisol at home

Mothers were asked to collect saliva samples at home from themselves and their children at three times, 30 min after waking up, at 2-3pm and just before bedtime. All control-mothers ($n=18$, 100%) and 18 IPV-PTSD (67%) complied. ANOVA repeated measures of mothers’ cortisol levels showed a significant effect for groupxtime interaction ($F_{2,68}=8.6$, $p<0.001$, $\eta^2_p=0.2$), and time ($F_{2,68}=51.4$, $p<0.001$, $\eta^2_p=0.6$), while group was not significant ($F_{1,34}=0.45$, $p>0.5$, $\eta^2_p=0.01$). Compared to control mothers, cortisol levels of IPV-PTSD mothers were significantly lower 30 min after awakening.
Several mothers reported problems collecting saliva samples from their children at home; 8 control (47%) and 12 IPV-PTSD (44%) mothers were successful at collecting all three saliva samples. ANOVA repeated measures of child cortisol levels at home showed a significant effect for time (Fig.2D; $F_{2,36}=18.7, p<0.001, \eta^2_p=0.51$). However, group ($F_{1,18}=0.33, p>0.6, \eta^2_p=0.02$) and groupxtime interaction ($F_{2,36}=2.7, p>0.8, \eta^2_p=0.02$) were not significant. In toddlers, morning cortisol levels were higher than cortisol levels at 2-3pm and bedtime cortisol $[t_{s(19)}=4.32-4.95, ps<0.001,d>1.16]$; but no significant differences were found between cortisol levels at 2-3pm and bedtime cortisol $[t(19)=1.86, p>0.08, d=0.52]$. In order to include the maximum number of toddlers in the analyses, we performed $t$-test analyses of cortisol samples at each point time. No significant differences were found between groups at any time point [Morning: HC=$12.7\pm2.1$, IPV-PTSD=$11\pm1.3$, $t(28)=0.8, p>0.44,d=0.28$; 2-3pm: HC=$8.1\pm0.6$, IPV-PTSD=$7.5\pm0.7$, $t(29)=0.47, p>0.64,d=0.17$; Night: HC=$5.6\pm0.4$, IPV-PTSD=$5.1\pm0.87$, $t(24)=0.46, p>0.65,d=0.19$].

Anticipatory anxiety

In order to assess whether simply participating in a laboratory session affected baseline cortisol levels of mothers and their toddlers, saliva samples were collected at home at a similar time as the baseline sample collection. Mothers’ baseline cortisol levels (saliva samples taken 10 min after arrival and prior to the laboratory session) were
significantly elevated compared to home cortisol levels at a similar time (2-3pm). This result suggests that mothers experienced anticipatory anxiety [cortisol-home=8±0.4; cortisol-baseline = 10.5±0.6; t(35)= -3.9, p<0.001, d=-0.73]. Analyses performed independently in each group showed significant differences in both groups [HC: cortisol-home=7.67±0.66; cortisol-baseline=10.6±0.97, t(17)= -2.5, p<0.021, d=-0.8; IPV-PTSD: cortisol-home=8.4±0.6; cortisol-baseline=10.7±0.97; t(17)= -3.04, p<0.01, d=-0.64].

Child afternoon home cortisol levels did not differ from child baseline laboratory-based cortisol levels among controls [cortisol-home=7.8±0.8; cortisol-baseline=6.7±0.54; t(7)= -1.2, p>0.28, d=0.55] or among children of IPV-PTSD mothers [cortisol-home=7.2±0.6; cortisol-baseline=8.7±0.4; t(11)=1.7, p>0.11, d=-0.75]. Cortisol data pooled from both groups together was not significant [cortisol-home=7.5±0.5; cortisol-baseline=7.9±0.4; t(19)=0.6, p>0.6, d=-0.18].

Mothers’ rating of anxiety levels

During the laboratory session, each time that saliva samples were collected, mothers were asked to rate their own anxiety levels and the anxiety levels of their children along a Likert scale 1-5 (1=no stress; 5=very stressed). No significant differences between groups were found with respect to mothers’ self-reported anxiety levels (Fig.3A; Mann-Whitney-test, n=45, U$s=258-310$, Z$s=0.57-1.76$, ps>0.08) or to mothers’ estimation of anxiety levels among their toddlers (Fig.3B; Mann-Whitney-test, n=45, U$s=234-300$, Z$s=-0.02-1.53$, ps>0.12).

In the group IPV-PTSD, mothers reported lower anxiety levels 30 min after the end of the laboratory stressor when compared to self-reported anxiety levels immediately after the stressor (Fig.3A; Wilcoxon-signed-rank-test, n=27, W=10, Z=-2.39, p<0.02, d=0.188). No significant differences were found in IPV-PTSD mothers self-reported anxiety levels at other time points (n=27, W$s=20-85$, Z$s=-1.16-1.53$, ps>0.12). Non-PTSD
control mothers self-reported anxiety levels did not significantly change during the session (Wilcoxon-signed-rank-test, \( n=18, W_s=0-16, Z_s=-1.4-0.45, ps>0.15 \)).

Control and IPV-PTSD mothers estimated that the anxiety levels of their children were significantly higher immediately after the stressor (T0) compared to baseline [HC, \( n=18, W=71, Z=2.55, p<0.02 \); IPV-PTSD, \( n=27, W=124.5, Z=2.3, p<0.02 \)], 30 min [HC, \( n=18, W=0, Z=-3.01, p<0.01 \); IPV-PTSD, \( n=27, W=27.5, Z=-2.97, p<0.01 \)], and 60 min [HC, \( n=18, W=0, Z=-3.12, p<0.01 \); IPV-PTSD, \( n=27, W=39, Z=-2.1, p<0.04 \)], after the stressor (Fig.3B). No significant differences were found on mother’s estimation of anxiety levels at other time points [HC, \( n=18, W_s=0-30, Z_s=-1.73-0.26, ps>0.08 \); IPV-PTSD, \( n=27, W_s=33-53.5, Z_s=-1.47-0.53, ps>0.14 \)].

Children's behavioral distress during stressors

Child behavior during the stressor segments (i.e., separation and exposure to novel stimuli) was assessed by independent trained observers. No significant group differences were found with respect to child-participants' behavioral display of distress during administration of laboratory stressors (Mann-Whitney-test, \( n=45, U_s=187-289, Z_s=-1.3-1.07, ps>0.19 \)).

Correlation between mothers’ estimation of anxiety and toddlers’ display of distress

Mothers’ estimation of the anxiety levels of their toddlers immediately after stress correlated significantly with total behavioral distress displayed by the toddlers in the control group (\( r_s=0.7, n=18, p<0.001 \)) and IPV-PTSD (\( r_s=0.5, n=27, p<0.009 \)) (Fig.3C, all mothers; \( r_s=0.6, n=45, p<0.001 \)).

Correlation between mothers’ difficulty identifying feelings and cortisol levels immediately after stressor
In a previous article, we already showed that IPV-PTSD mothers have an increase in alexithymia, especially in the subscale of identifying feelings in self and other. Taking into account only the dyads included in the present analyses (with the exception of 4 mothers who did not fill the questionnaire), we found that IPV-PTSD mothers had increased difficulty identifying emotions (Mann-Whitney-test, \( n=41, U=256, p<0.018 \)). Interestingly, mothers’ cortisol levels immediately after stress correlated negatively with maternal difficulty in identifying emotions in self and other on the Toronto Alexithymia Scale (Fig.4, \( r_s=-0.33, p<0.04, n=41 \)).

Discussion

Diurnal cortisol and cortisol stress reactivity are of particular interest in understanding the potential effects of IPV-PTSD on maternal and child behavior. The current findings showed that mothers with IPV-PTSD as compared to HC had lower cortisol levels in response to the laboratory stressor, which included child-parent separation; this being the case even though distress behavior of the children and mothers’ rating of toddler’s anxiety was similar between groups. Importantly, children of IPV-PTSD mothers showed a blunted cortisol response to stress when compared to children of HC mothers. In comparison to controls, IPV-PTSD mothers had lower morning and higher nighttime cortisol levels. Diurnal cortisol release was not affected among children with IPV-PTSD mothers.

Mothers’ cortisol

In both groups, cortisol levels followed a circadian pattern with higher levels in the morning that declined throughout the day (Debono et al., 2009). As expected, IPV-PTSD mothers had lower morning cortisol levels than HC mothers. Previous studies have shown that PTSD that is linked to chronic, particularly early onset exposure to traumatic
experiences, such as child maltreatment and exposure to domestic violence (Griffin et al., 2005), as it is the case for the mothers in the present study, is associated with reduced morning cortisol levels [for review see (Daskalakis et al., 2013)]. This was similarly the case among mothers affected by Holocaust-related trauma (Yehuda et al., 2005).

It is important to mention that reduced morning cortisol levels is not an exclusive endocrine marker for PTSD, as for example, a similar pattern of hypocortisolemia has been reported, among others, in patients with severe depression with psychotic features (Gold and Chrousos, 1999; Gonul et al., 2016) [as opposed to patients with melancholic depression that show elevated diurnal cortisol levels (Holsboer, 2001)], and low morning cortisol has been linked to criminal and aggressive behavior (Cima et al., 2008). Furthermore, not all the studies have reported low cortisol levels in PTSD, there have been some exceptions [for review see (Yehuda, 2009)]. Nevertheless, animal models that use PTSD-relevant fear-conditioning models have found that exposure to traumatic experience reduced glucocorticoids release (Zoladz et al., 2012; Zoladz et al., 2015). Importantly, in a rat model for IPV, female rats cohabitating with aggressive male partners developed a plethora of symptoms that were highly pertinent to human PTSD (increased anxiety, hypervigilance, social avoidance, etc.) accompanied by a reduction of basal glucocorticoid levels (Cordero et al., 2012).

Furthermore, in response to their child’s distress (i.e. following separation), IPV-PTSD mothers’ cortisol levels were lower than those of HC mothers, even though maternal subjective appraisal of their toddlers’ distress to the stressors, and subjective rating of their own distress were not different between groups. Several studies have shown a blunted cortisol response to acute stress in individuals with prior exposure to chronic, extreme stress, such as child physical abuse (Harkness et al., 2011; MacMillan et al., 2009; Ouellet-Morin et al., 2011). Additionally, PTSD patients most typically present a
blunted cortisol-response to stress that is possibly due to a strong negative feedback loop (Morris et al., 2012; Yehuda, 2009). Increased receptor sensitivity and negative glucocorticoid-feedback have also been reported among IPV-PTSD women (Griffin et al., 2005). In comparison to a normative sample, subjects with lifetime IPV-PTSD have shown i) lower morning cortisol, ii) increased mRNA expression of glucocorticoid receptors and iii) lower glucocorticoid receptor gene methylation; importantly GR gene methylation was found to correlate negatively with mRNA GR expression (Labonte et al., 2014). In line with these results, our group has reported a reduction of the methylation status of the glucocorticoid receptor gene (NR3C1) in another cohort of IPV-PTSD women from the same study (Schechter et al., 2015a). Although still understudied, NR3C1 methylation has been pointed to as a key element involved on the long-term stress effects on HPA-axis function [for review, (Palma-Gudiel et al., 2015)].

Importantly, in the context of mother-child interaction, low maternal cortisol reactivity in response to child distress may negatively impact maternal behavior. Cortisol in response to stress has been shown to enhance amygdala connectivity along with the medial prefrontal cortex and other areas critically involved in emotional processing (Quaedflieg et al., 2015). Thus, cortisol reactivity may be linked to the facilitation of the identification of facial expression of emotion (Adolphs, 2010; Schulte-Ruther et al., 2007). Along those lines, we found in the present study that maternal cortisol levels following the laboratory stressor were negatively correlated with maternal difficulty in identifying emotions on a measure of alexithymia. Though, the results may seem contradictory in that IPV-PTSD mothers have difficulty identifying emotions but remain dysregulated by their toddlers’ distress, it is important to highlight that in the present study we did not ask the mothers to identify the specific emotion of the child. In a previous publication, Schechter et al. (2004) showed that IPV-PTSD mothers were more inclined
to attribute the distress behaviors of the toddlers (e.g., crying) to feeling “angry” or “hostile” or “controlling” rather than feeling “fearful and helpless” or “in need of comfort” (e.g., toddlers’ cries in one paper were attributed to trying to stop or punish the mother for leaving the room [(Schechter and Rusconi-Serpa, 2013)]). Accordingly, we had previously reported data drawn from the same sample, that this difficulty in identifying emotions is associated with less maternal sensitivity among IPV-PTSD mothers (Schechter et al., 2015b). Moreover, we found, also in this same cohort, that lower NR3C1 gene methylation was associated with reduced activity in the maternal medial prefrontal cortex (mPFC) [which has been noted to be a key brain area implicated in emotion processing, (Phillips et al., 2003)], in response to video-stimuli of mother-child separation versus play (Schechter et al., 2015a). Altogether, the evidence suggests a link between maternal IPV-PTSD and HPA-axis dysregulation that is characterized by hypocortisolism and low cortisol reactivity, that is additionally associated with i) reduced activation of emotion processing brain areas, and ii) parenting impairment in response to child stress.

Children’s cortisol

As shown in previous research (Watamura et al., 2004), toddlers ages 1- to 4-years-old show significantly decreased cortisol levels from morning to night-time sampling. Contrary to our expectations that we would find low cortisol in offspring of PTSD mothers as had been the case in prior studies (Bader et al., 2014; Lehrner et al., 2014; Yehuda et al., 2008; Yehuda and Bierer, 2008), toddlers of IPV-PTSD mothers did not differ from control toddlers in diurnal cortisol pattern release. A possible explanation for this difference is that diurnal cortisol release effects depend on the time of exposure of the offspring to the trauma related to development of maternal PTSD, pregnancy being
of key relevance for epigenetic effects on glucocorticoid programming (Bader et al., 2014; Yehuda and Bierer, 2008).

On the other hand, we found that children of IPV-PTSD mothers had a blunted cortisol response to stress by ages 12-42 months. Our results are in line with other reports in the literature that show that risk factors such as very low-income and high maternal depressive symptoms (Fernald et al., 2008), IPV and maternal emotional unavailability (Sturge-Apple et al., 2012) are related to low cortisol stress response in young children (ages of 2 - 6 years). It has been proposed that increased cortisol levels during stress response suppresses fear conditioning and reduces amygdala activity, preventing the latter from being over-activated (Sapolsky et al., 2000). Therefore, low cortisol release in response to stress, especially during early development, might increase the risk to develop future anxiety disorders. Enhanced sensitivity and negative glucocorticoid feedback has been reported in PTSD offspring (Lehrner et al., 2014), and it has been proposed as a mediator factor of increased risk to develop PTSD (Yehuda et al., 2008). Additionally, low salivary cortisol levels have been related to aggressive behavior in young boys (Shirtcliff et al., 2005; Shoal et al., 2003) and development of antisocial behavior (Gostisha et al., 2014; Shirtcliff et al., 2009).

To the best of our knowledge, this study is the first to assess mothers and their toddlers together, including non-PTSD controls and IPV-PTSD cases, and both physiological (i.e. cortisol) and psychological distress (i.e. maternal distress and maternal estimation of their toddlers’ distress); as well as the evaluation of toddlers’ distress by trained observers, under the conditions of a laboratory stress paradigm. While many studies only assess cortisol stress reactivity or circadian rhythm, we assessed both together in the same population of mothers and toddlers, thus providing more complete information regarding the HPA-axis function (De Kloet, 2004). The protocol was
carefully designed in order to collect all the relevant information, and it was informed by and built upon the findings of a previous study (Schechter et al., 2004). In the previous study, during the laboratory session, only maternal cortisol was measured (not toddlers’ cortisol), and importantly, saliva was collected only 30 min after separation, therefore the findings were compared neither with cortisol baseline levels in the laboratory before the separation, nor with baseline levels at home. No information regarding the behavioral distress of the toddlers, or maternal estimation of toddlers’ behavioral distress, was collected during the session. Another important difference with the previous study is that the participant sample in the previous study only included mothers with a lifetime of IPV-PTSD and no control-group. The findings provided in the present paper, therefore, provide key information from our longitudinal study of the effects of IPV and related maternal psychopathology on developmental psychopathology of the child. The latter allowed the combination and integration of relevant findings that had been reported in previously published work (Moser et al., 2015a; Moser et al., 2015b; Moser et al., 2015c; Schechter et al., 2015a; Schechter et al., 2016; Schechter et al., 2015b) with those of maternal and child HPA-axis function within the same sample of participants.

**Limitations**

One of the limitations of our study was the fact that IPV-PTSD mothers had lower SES than non-PTSD controls. It is possible that the proportion of IPV-PTSD women of low SES is higher because poverty is associated with higher rates of violence. It is also possible that this confounder is related to sample bias in that lower SES mothers with IPV-PTSD were willing to participate in this remunerated study than higher SES mothers. Low SES is in and of itself, a powerful chronic stressor that has been linked to disrupted neuroendocrine regulation and impaired health [for review, (Seeman et al., 2010)]. Nonetheless, it was beyond the scope of this study to discern whether lower SES was
more of an effect of psychopathology or a risk factor for it (Koenen et al., 2007; Pinto Pereira et al., 2016)

Another limitation of our study was the small sample-size that precluded us from assessing toddler gender and age effects on the outcomes. That being said, studies of cortisol levels among very young children have not thus far found significant gender differences (Bright et al., 2014).

Conclusions

Consistent with the literature, we found that IPV-PTSD mothers showed significantly lower cortisol levels during a laboratory stressor and lower morning and higher bedtime salivary cortisol levels than non-PTSD controls. Similarly, the children of the IPV-PTSD mothers showed significantly lower reactivity to the laboratory stressors than those of non-PTSD controls, while no differences were found on diurnal cortisol release. This study is the first to our knowledge to show this psychophysiological dysregulation within the HPA-axis for mothers with IPV-PTSD and their toddlers as compared to non-PTSD controls. Further research within larger samples is needed to understand better, individual differences in HPA-axis response to stress in this at-risk population as these differences might be related to subsequent developmental psychopathology and to response to parent-child intervention.

Conflict of interest

Authors have no conflict of interest to disclose.

Acknowledgements

This research was supported by the National Center of Competence in Research (NCCR) “SYNAPSY - The Synaptic Bases of Mental Diseases” financed by the Swiss National Science Foundation (n° 51AU40_125759), the Gertrude von Meissner Foundation, the
Oak Foundation and la Fondation Prim’Enfance. This work was further supported in part by the Center for Biomedical Imaging (CIBM) of the Geneva–Lausanne Universities, the EPFL, and the Geneva–Lausanne University Hospitals.
References


Cima, M., Smeets, T., Jelacic, M., 2008. Self-reported trauma, cortisol levels, and aggression in psychopathic and non-psychopathic prison inmates. Biol Psychol 78, 75-86.


Largo, R.H., Pfister, D., Molinari, L., Kundu, S., Lipp, A., Duc, G., 1989. Significance of prenatal, perinatal and postnatal factors in the development of AGA preterm
infants at five to seven years. Developmental medicine and child neurology 31, 440-456.


**Figure 1.** Timeline and schema of saliva collection during the laboratory stressor. Saliva samples from mothers and children at different time points were collected to study cortisol stress reactivity. The first saliva collection was collected 10 min after arrival (baseline), the second immediately at the end of the last stressor (T0), and the last two samples at 30 (T30) and 60 (T60) min afterwards.

**Figure 2.** Salivary cortisol stress reactivity during the course of the laboratory session in mothers (A) and their toddlers (B); IPV-PTSD mothers and their children showed blunted cortisol reactivity to laboratory stress. Cortisol levels at home in mothers (C) and toddlers (D); compared to non-PTSD controls, IPV-PTSD mothers (but not their toddlers) had lower morning and higher bedtime cortisol levels. Data are presented as the mean ± S.E.M. *, p<0.05.

**Figure 3.** Self-reported anxiety levels of mothers (A) and mothers’ estimation of anxiety levels of the children (B). Correlation between mothers’ estimation of the anxiety levels of their toddlers immediately after stress and total behavioural distress displayed by the toddlers (C). Data are presented as the mean ± S.E.M. Wilcoxon-signed-rank-test: $*, p<0.05$ vs 30 min after end of the laboratory stressor. Mann-Whitney test: #, p<0.05, vs baseline, 30 min and 60 min after stressor.

**Figure 4.** Correlation between mothers’ cortisol levels immediately after stress (T0) and maternal difficulty in identifying emotions in self and other on the Toronto Alexithymia Scale.
PROTOCOL SALIVA SAMPLE COLLECTION

**Activities**

<table>
<thead>
<tr>
<th>Arrival</th>
<th>Free Play</th>
<th>Reunion/ Play</th>
<th>Tiding up</th>
<th>Structured activity</th>
<th>Reunion</th>
<th>Stressors</th>
<th>Saliva samples (cortisol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 min</td>
<td>3 min</td>
<td>2 min</td>
<td>2 min</td>
<td>4 min</td>
<td>3 min</td>
<td>2 min</td>
<td>3 min</td>
</tr>
<tr>
<td>30 min</td>
<td>30 min</td>
<td>2 nd Separation</td>
<td>Clown</td>
<td>Scary toys</td>
<td>T0</td>
<td>T30</td>
<td>T60</td>
</tr>
<tr>
<td>Stressors</td>
<td>Baseline</td>
<td>30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Stressors**

1st Separation

2nd Separation

Clown

Scary toys
A) Mothers

Mothers' self-reported anxiety over time after stress.

B) Toddlers

Mothers' estimation of toddler anxiety over time after stress.

C) Correlation

Correlation between toddlers' distress and mothers' estimation of anxiety. The Spearman's rank correlation coefficient ($r_s$) is 0.6, with $p<0.001$. 
$r_s = -0.33$, $p < 0.04$