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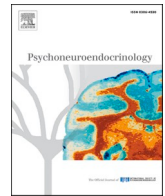
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# Preliminary findings on the association between maternal salivary and hair cortisol and the mother-infant-interaction during the early postpartum period

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## ABSTRACT

Maternal capabilities to engage in sensitive caregiving are important for infant development and mother-infant-interaction, however, can be negatively affected by cortisol due to a stress response. Previous research suggested that cortisol possibly impairs cognitive functions important for caregiving behavior, which potentially leads to less maternal sensitivity. However, studies investigating the influence of cortisol using endocrine parameters on the mother-infant-interaction during the early postpartum are lacking. In the current study, fifty-nine mother-infant-dyads participated in a laboratory face-to-face still-face (FFSF) observation when infants were 4 months of age. Maternal and infant positive, negative and matched behavior during the FFSF was microanalytically coded. Cortisol concentrations were obtained using hair and saliva samples. For salivary cortisol, the area under the curve with respect to ground (AUC<sub>G</sub>) was calculated using two saliva samples obtained after arrival and after the FFSF. Multiple block-wise hierarchical linear regression models were conducted to incorporate potential confounding factors (maternal age, parity, infant gestational age, infant sex) in a first step and, then, test for the association of hair and salivary cortisol with maternal and infant positive, negative and dyadic behavior in a second step. For both it was hypothesized that cortisol assessed in hair and saliva is negatively associated with positive and matched mother-infant-interaction, and positively associated with negative mother-infant-interaction. It could be shown that salivary but not hair cortisol as well as infant gestational age and infant sex related significantly to infant positive and negative affect as well as matched behavior during the reunion phase of the FFSF. Maternal positive affect was unrelated to any of the variables. The results are discussed in regard to the importance of maternal cortisol levels over a longer period of time and more acute situational levels for the mother-infant-interaction as well as the relevance of included confounding factors.

## 1. Introduction

The mother-infant-interaction is – besides the interaction with other relevant caregivers – one of the first experiences of newborn infants and crucial for infant's brain development (Ilyka et al., 2021). The mother serves in a coregulating function as infants cannot yet fully cope with different stimuli (Laurent et al., 2016), and enables the development of self-regulation strategies (Blair and Ku, 2022). For this, however, the mother needs to adequately perceive, interpret and react to the infant's signals, a skill called maternal sensitivity (Bowlby and Ainsworth, 2000;

Shin et al., 2008). Women transitioning to motherhood face crucial changes which could potentially be stressful for the mother (Emmanuel and St John, 2010). Although, evidence suggests a negative effect of psychosocial stress on maternal sensitivity during mother-infant-interactions (Almanza-Sepulveda et al., 2020; Booth et al., 2018; Finegood et al., 2016), the effects of cortisol, a hormone that is released by the hypothalamus pituitary adrenal (HPA) axis and can also be associated with psychosocial stress (Smith and Vale, 2006; Tsigos et al., 2020), on maternal abilities to engage in sensitive caregiving have not been extensively examined.

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The HPA axis regulates different bodily functions and underlies a diurnal rhythm where cortisol levels rise right after waking up and then progressively decrease during the course of the day (Bhake et al., 2019; Ranjit et al., 2005). In the context of an occurring stressor, the HPA axis is activated (further referred to as the “stress response”) and the hypothalamus releases corticotropin releasing hormone, which induces the release of adrenocorticotrophic hormone from the pituitary gland, ultimately causing the secretion of cortisol by the adrenal gland into the body, which prepares the individual for a subsequent stress response (e.g., fight or flight; Cole and Kramer, 2016). The secretion of cortisol following a stress response is typically terminated due to a negative feedback loop that regulates the synthesis of cortisol (Smith and Vale, 2006). If a stressor persists a dysregulation or even alteration of the HPA axis activity and the diurnal cortisol slope have been reported as a possible consequence (McEwen, 2004; Sjögren et al., 2006; Tsigos et al., 2020).

It has been discussed that cortisol affects different cognitive systems, specifically, when elevated following a stressor. Plessow et al. (2011) and Goldfarb et al. (2017) discussed the effect of cortisol elevation following a stressor on cognitive flexibility. Plessow et al. (2011) found that cortisol led to a decrease in cognitive flexibility and stressed individuals had more difficulties compared to non-stressed individuals to include new information in already existing information, while Goldfarb et al. (2017) found that stressed individuals had more difficulties to shield themselves from distractors. Whereas Plessow et al. (2011) used a psychosocial stress task, the Trier Social Stress Test (Kirschbaum et al., 2008), Goldfarb et al. (2017) used a cold pressure task. Although both stress tasks can evoke a cortisol elevation, it has been discussed that the elevation of HPA axis activity due to a stress response seems to be more sensitive towards psychosocial stressors (Pruessner and Ali, 2015). However, both studies found that stressed individuals did not show worse overall performance compared to non-stressed individuals (Goldfarb et al., 2017; Plessow et al., 2011). Schwabe and Wolf (2013) discussed that cortisol elevation possibly affects memory systems that ultimately lead to a shift from cognitive (i.e., sensitive) to more habitual memory systems. As the authors note, this may lead to less goal-directed behavior, which can affect behavioral flexibility, however, can also be associated with a less demanding memory function which allows the individual to use the free resources to cope with an occurring stressor (Schwabe and Wolf, 2013). Mother’s ability to sensitively interact with an infant potentially relies on a manifold of factors. As defined by Shin et al. (2008), maternal sensitivity relies on maternal perception and interpretation of infant cues as well as on an appropriate response in a contingent manner. Further, sensitive mothers should be able to adapt and change these processes during the mother-infant-interaction, which makes maternal sensitivity a highly dynamic construct (Shin et al., 2008). Based on this definition, it is likely that cortisol can affect cognitive processes that are relevant for maternal sensitivity. Further, if an individual cannot cope with a stressor over a longer period leading to a dysregulation of the HPA axis, an alternated responsiveness of the HPA axis can be the consequence which can be associated with various risks for mental health problems and psychiatric disorders (Hammen, 2005; McEwen, 2004).

Based on the aforementioned literature (Goldfarb et al., 2017; Plessow et al., 2011; Schwabe and Wolf, 2013) cortisol can possibly also impair maternal capabilities to engage in sensitive caregiving, specifically, at the cost of cognitive and behavioral flexibility. However, flexibility could be crucial for mothers looking at some important demands during dyadic interaction. The mother-infant-interaction can be characterized by states of matches and mismatches between both interaction partners (DiCorcia and Tronick, 2011). During matching states, maternal regulatory input aligns with infant regulatory needs, while failing to do so can lead to mismatching states (DiCorcia and Tronick, 2011). At early infancy, maternal sensitivity helps the mother to adequately repair the interaction and change back to matching states when infants are not capable to regulate themselves (DiCorcia and

Tronick, 2011; Gianino and Tronick, 1988). The effective dyadic reparation after a mismatching state fosters infant’s own coping capabilities and promotes the development of self-regulatory strategies, whereas ineffective reparation potentially stresses the infant which is assumed to hinder infants’ socio-emotional and cognitive development (Braungart-Rieker et al., 2014; DiCorcia and Tronick, 2011). Maternal sensitivity could predict infant’s responses to stressors and dyadic regulation in standardized paradigms like the face-to-face still-face (FFSF; Mesman et al., 2009). During the FFSF, caregivers provoke a mismatching state making it possible to examine the infant’s reaction and dyadic patterns of regulation (Mesman et al., 2009; Tronick et al., 1978). As a reaction to the caregiver’s still-face, the infant reliably increases negative affect, protesting and self-comforting behavior while decreasing positive affect (the so-called still-face effect), and the dyadic regulation can be reflected in a change back to affective states comparable to the play phase after the mother-infant-interaction is resumed (Mesman et al., 2009). It could be shown that infant’s responses and dyadic regulation can be associated with the quality of attachment (Müller et al., 2022), maternal sensitivity (Braungart-Rieker et al., 2014), and psychosocial stress (Tronick et al., 2021). Likewise, elevated cortisol levels over a longer period of time may be associated with maternal mental health problems, like depression or anxiety, which have been shown to affect the quality of mother-infant-interactions (Feldman et al., 2009) and maternal sensitivity (Stanley et al., 2004; Tester-Jones et al., 2016).

There are only few studies that examined the association between maternal cortisol and maternal sensitivity during mother-infant-interactions in the early postpartum period. During pregnancy until 3 months postpartum, maternal cortisol levels are generally higher compared to non-pregnant women, and change back to normal levels after 3 months postpartum (Almanza-Sepulveda et al., 2020). In humans, higher cortisol levels shortly after birth could be associated with higher alertness for infant cues, however, after 3 months postpartum, higher levels of cortisol could be associated with less optimal caregiving and sensitivity in mothers (Almanza-Sepulveda et al., 2020), with the latter possibly adding to the existing literature on the effects of elevated cortisol in the context of a stress response. Studies considering salivary cortisol concentrations (SCC) as a marker for situational cortisol levels (Kirschbaum and Hellhammer, 2008) during mother-infant-observations are scarce. Thompson and Trevathan (2008) found that maternal cortisol elevation during a free-play observation led to less synchronous behavior between mothers and their 3-month-old infants, which was interpreted as less maternal sensitivity by the authors. Finegood et al. (2016) found that mothers who experienced adverse life events showed a negative association between cortisol levels and maternal sensitivity during the first 2 years after birth during free-play observations at 7 and 15 months of age and a puzzle-task at 24 months of age. However, these studies slightly differ regarding the assessment of salivary cortisol, with Thompson and Trevathan (2008) using two measures during the observation and calculating the difference between the obtained cortisol levels to index a cortisol increase or decrease, whereas Finegood et al. (2016) obtained three samples over the course of the experiment and calculated the average of all samples. Further, none of these studies used the FFSF during observations, although it allows a more standardized observational method to assess maternal sensitivity. Another study indicated that a maternal stressor prior to the FFSF potentially leads to more negative interaction patterns between the mother and the infant, however, did not control for maternal cortisol levels following the stress manipulation (Mueller et al., 2021; Tronick et al., 2021). Using human hair for the examination of cumulated cortisol levels (Meyer and Novak, 2012), Khoury et al. (2020) found higher hair cortisol concentrations (HCC) during pregnancy and depressive symptoms of the mother related to maternal withdrawal and intrusiveness during the FFSF with 4-month-olds. Tarullo et al. (2017) found a negative association between maternal postpartum HCC and positive engagement, and a positive association with maternal

intrusiveness during the FFSF with 5–7-months-olds. In sum, both long-term as well as situational cortisol levels seem to be important factors that can affect the mother-infant-interaction. To date no prior study investigated both markers, HCC as a marker for cumulated cortisol levels and SCC as a marker for situational cortisol levels, and the mother-infant-interaction during the FFSF.

In sum, the mother-infant-interaction is important for the socio-emotional development of infants and is affected by maternal abilities to engage in sensitive caregiving. Although, maternal cortisol levels possibly affect these maternal abilities and related cognitive systems (Almanza-Sepulveda et al., 2020; Booth et al., 2018; Goldfarb et al., 2017; Plessow et al., 2011; Schwabe and Wolf, 2013), few studies investigated the influence of situational and long-term cortisol levels, in the early postpartum period. Endocrine markers can be used to assess situational and long-term cortisol levels as indicators for overall HPA axis activity as well as for a stress response (Hellhammer et al., 2009; Kirschbaum and Hellhammer, 2008; Stalder and Kirschbaum, 2012; Stalder et al., 2017) and significant associations between SCC and HCC with maternal sensitivity or the quality of mother-infant-interactions have been found (Finegood et al., 2016; Khoury et al., 2020; Nystrom-Hansen et al., 2019; Tarullo et al., 2017; Thompson and Trevathan, 2008). The current study aims to investigate the association between maternal situational and cumulated cortisol levels with the mother-infant-interaction during the FFSF in a sample of mother-infant-dyads 4 months postpartum. The FFSF provokes a mismatching state between mothers and their infants. During the reunion, mothers resume the normal interaction and dyadic regulation can be observed as a consequence of the mismatching state. The first part of our study analyzed the so-called still-face effect with the assumption that infants react to the mismatching state with less positive affect, more negative affect and more self-comforting behavior while mothers maintain a still-face. During the last phase of the FFSF, it is proposed that infants negative and positive affect as well as self-comforting behavior resumes to levels comparable to the start of the FFSF. To reflect maternal cortisol, two measures were used. SCC was assessed and should reflect more momentary maternal cortisol levels during the interaction with her infant. HCC was used to reflect long-term, cumulated maternal cortisol levels during the past 3 months. Both measures are therefore related to activity of the HPA axis and evidence suggests moderate to large correlations between SCC and HCC (Short et al., 2016; Singh Solorzano et al., 2023; Zhang et al., 2018). The mother-infant-interaction is operationalized by different maternal, infant and dyadic behaviors assessed with the Infant and Caregiver Engagement Phases Revised (ICEP-R; Reck et al., 2009). The ICEP-R allows to individually assess maternal affect, infant affect and the dyadic behavior of mothers and infants (Reck et al., 2009). In the current study, higher SCC and HCC are both hypothesized to be associated with less positive infant and maternal affect, more negative infant affect, less matched dyadic behavior, which was operationalized as shared positive affect and eye-contact, as well as with a longer delay of dyadic regulation after maternal unresponsiveness during the FFSF indicated by shared positive affect.

## 2. Methods

The current study is part of a larger monocentric, prospective cohort study, which is registered in the German Clinical Trials Register (number DRKS00024921). Data collection occurred between August 2021 and January 2022 at the Institute of Experimental Psychology at the Heinrich-Heine-University Düsseldorf. The study protocol was approved by the local ethics committee (No. 2021–1329) and in accordance with the declaration of Helsinki. The overall study aims to investigate the differential effects and interactions between maternal and infant cortisol and maternal perceived stress as well as maternal mental health on the mother-infant-interaction as well as bonding with a specific focus on differences between mothers with full-term and preterm infants. As

recruitment of the preterm mothers was far more difficult than anticipated, data collection of the overall study is currently still ongoing. The current work is a secondary analysis of the whole data set that only includes full-term infants and their mothers. Further, the current work focuses only on maternal cortisol measures and the association with the mother-infant-interaction.

### 2.1. Recruitment and participants

Mother-infant-dyads were recruited via the residents' registration office of the city of Düsseldorf, Germany. Members of the study group contacted mothers who indicated interest in study participation via telephone and informed them about the purpose and procedure of the study. All mothers had to be fluent in German and over 18 years old. Infants had no serious illness or congenital developmental disorders. Dyads were invited when infants were 4 months of age (see Table 1 for mean age). All legal guardians of infants gave their written informed consent for study participation, data recording and storage and received a travel refund as well as a present for participation at the end of the experiment.

In total,  $N = 76$  mothers with full-term infants participated in the study. Of these dyads,  $n = 8$  had to be excluded due to technical issues or a deviation from the study protocol,  $n = 5$  had to be excluded as infants expressed crying or protesting behavior  $\geq 80\%$  of the overall time during the FFSF, and of  $n = 1$  dyad the corresponding SCC could not be analyzed, leaving a sample of  $n = 62$  dyads. For  $n = 3$  dyads, the corresponding HCC was regarded as a statistical outlier, as well as for  $n = 1$  of these the corresponding SCC, and were not included in the corresponding analyses, leaving a final sample of  $n = 59$  for data analysis. All

**Table 1**  
Overview of maternal and infant demographic variables.

	<i>N</i>	<i>M</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>
<b>Mothers</b>					
Age (in years)		35.41	4.32	26	43
Nationality					
German	52				
Other	7				
Marital status					
Married/registered partnership and living together	44				
Married/registered partnership and not living together	1				
Living with a partner	9				
Single	5				
Education					
Technical diploma	1				
High-school diploma	10				
University degree	41				
Doctorate	7				
Parity					
Primiparous	31				
Multiparous	28				
<b>Infants</b>					
Sex assigned at birth					
Male	31				
Female	28				
Gestational age at birth (in days)		277.58	8.64	262	292
Age at study participation (in days)		127.12	6.52	111	143
Birth weight (in g) <sup>a</sup>		3492.67	388.95	2640	4460
Birth height (in cm) <sup>a</sup>		52.06	2.06	48	57
Birth mode					
Vaginal	41				
C-section	12				
Assisted	6				

Note:  $N = 59$  mother-infant-dyads; <sup>a</sup> data of  $n = 58$  due to missing information of one dyad.



sociodemographic variables of included dyads are reported in [Table 1](#).

## 2.2. Materials and measures

### 2.2.1. Salivary cortisol concentrations

Salivary cortisol concentrations were measured for both mothers and infants and were assessed at three time points during the assessment: right at the beginning before behavioral observations (T0), right after behavioral observations (T1), and approximately 25 minutes after T1 (T2). For the current study, only maternal SCC of T0 and T1 were of interest. Assuming a latency of approximately 20 minutes ([Kirschbaum and Hellhammer, 2008](#)), T0 should reflect the baseline cortisol levels of mothers before the examination, T1 approximately at arrival, and T2 right after the mother-infant-observation. Therefore, T0 and T1 should reflect maternal cortisol levels at the beginning of the examination, and should be unaffected by any circumstances that occurred during the FFSF (e.g., infant crying). For analysis, the area under the curve with respect to ground (AUC<sub>G</sub>) was calculated based on the recommendations of [Pruessner et al. \(2003\)](#). The AUC<sub>G</sub> reflects a time-adjusted mean of two measurements which should reflect maternal baseline cortisol levels prior and during the FFSF. The interval in minutes between T0 and T1 was  $M = 20.51$  ( $SD = 9.11$ ), and between T1 and T2  $M = 32.60$  ( $SD = 9.54$ ). Mothers were instructed to thoroughly insalivate a cotton swab. For the infants, mothers held the cotton swabs in their infants' mouth to collect saliva until the cotton swab was visibly insalivated. Saliva samples were frozen and stored at  $-20$  degrees Celsius until analysis. Analyses were carried out at Dresden LabService GmbH, Technical University of Dresden, Germany. After thawing, samples were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. SCC were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International GmbH, catalogue number R62111). The intra and inter assay coefficients of variance were below 9 %. Cortisol concentrations are reported in nmol/l. Mothers had a mean SCC at T0 of  $M = 3.52$  nmol/l ( $SD = 2.56$  nmol/l, range 0.73–14.47 nmol/l), T1 of  $M = 3.31$  nmol/l ( $SD = 2.28$  nmol/l, range 0.63–10.89 nmol/l), and T2 of  $M = 2.51$  nmol/l ( $SD = 1.60$  nmol/l, range 0.59–9.48 nmol/l). As free cortisol substantially fluctuates during the course of the day ([Bhake et al., 2019](#); [Ranjit et al., 2005](#)), time of day is an important factor while investigating SCC. Given a diurnal slope with highest cortisol levels shortly after waking up and a decline approximately at midday, time of day of sampling will be incorporated in the current study. The analyses of interest were tested again incorporating time of day as a dummy coded variable with '0 = before 12 pm' and '1 = after 12 pm'. As time of day as an additional factor did not change the initial findings, results can be seen in the [supplementary material](#) (see [Tables S5–S10](#)).

### 2.2.2. Maternal hair cortisol concentration

To assess maternal cumulative cortisol levels via HCC, a standardized protocol for hair collection and storage was used ([Meyer et al., 2014](#)). At the end of the experiment, the hair was divided from a posterior vertex position and, using a loop, an approximately 3 mm thick hair strand was tied off. The strand was ultimately cut off closely to the scalp and wrapped in tin foil. All samples were separately stored in air-tight bags. Until analysis, all samples were stored dark and at room temperature. Analyses were carried out by the laboratory of the Department of Cognitive Psychology at the University of Bochum. Hair strands were washed, dried, and grinded prior to cortisol extraction. The exact method can be seen elsewhere ([Meyer et al., 2014](#)). For analysis, the commercially available chemiluminescence immunoassay with high sensitivity (IBL International GmbH, catalogue number R62111) was used. The intra and inter assay coefficients of variance are reported to be 0.4–1.7 % and 0.8–1.8 % respectively ([IBL International GmbH, catalogue number R62111](#)). Human hair has an average growth rate of 1 cm per month and the cortisol concentration obtained refers analogously to one month ([Meyer et al., 2014](#)). In the current study, the first 3 cm were

analyzed and refer to the cumulated HCC during the past 3 months in pg/mg. Mothers had a mean HCC of  $M = 4.19$  pg/mg ( $SD = 2.20$  pg/mg, range 1.40–11.36 pg/mg).

### 2.2.3. Coding of maternal affect, infant affect and dyadic behavior

The mother-infant-observations were analyzed using Mangold INTERACT software and the behavior was coded according to the German version of the Infant and Caregiver Engagement Phases Revised (ICEP-R; [Reck et al., 2009](#)) by two trained raters. It allows to micro-analytically and separately code infant and maternal behavior as well as dyadic behavior during the interaction. Infant behavior can be coded as negative engagement (i.e., withdrawn and protest), object/environment engagement, social monitor, and social positive engagement. Maternal behavior can be coded as negative engagement (i.e., withdrawn, hostile and intrusive), non-infant focused engagement, social monitor/no vocalizations or neutral vocalizations, social monitor/positive vocalizations, and social positive engagement. Dyadic behavior was coded as dyadic eye-contact and joint activity/looking. Behavior was coded in seconds, indicating the total amount of time during the phases the respective code was shown. Infant, maternal and dyadic codes were mutually exclusive. Furthermore, additional information about self-comforting behavior (oral and self-clasp), distancing, and autonomic stress indicators of the infant as well as rough touches or violations of the FFSF of the mother were coded. For each code (except for *interactive repairation* which is reported in seconds), the total duration during a phase was then divided by the total length of the phase indicating the relative amount of the exhibited behavior during the respective phase. Raters were trained to reach 80 % reliability prior to coding. Ten videos were randomly selected to test for inter-rater-reliability and were coded by an additional third coder. For infant codes, the time-unit kappa was .76–.78 (agreement 86–88 %), and event-alignment kappa was .51 (agreement 79 %). For maternal codes, time-unit kappa was .50–.54 (agreement 63–66 %), and event-alignment kappa was .39 (agreement 66 %). Dyadic codes reached a time-unit kappa of .69–.70 (agreement 92 %), and event-alignment kappa of .56 (agreement 81 %). [Table 2](#) gives a brief description of the behavior of interest for the current study. In any cases where raters had difficulties or were uncertain about which code to assign in mother-infant-observations, the team of raters and the coding trainer discussed these instances to determine the appropriate code for the respective behavior observed in the video. For all codes, the relative amount of exhibited behavior was used for analysis, that is the total duration of the behavior during a phase was divided by the total length of the respective phase. See [supplemental Table S 1](#) for the duration of used codes in means and standard deviations.

### 2.2.4. Maternal depressive symptoms

Maternal depressive symptoms were assessed using the German version of the Edinburgh Postnatal Depression Scale (EPDS; [Bergant et al., 1998](#)) as part of the questionnaire mothers filled out after the video observation. The EPDS was originally developed by [Cox et al. \(1987\)](#) to assesses maternal depressive symptoms on ten items that can be answered on a 4-point Likert-scale (0–3) and are summed up to a total score (range 0–30) with higher scores indicating more severe maternal depressive symptoms ([Bergant et al., 1998](#); [Cox et al., 1987](#)). Different cut-off scores have been discussed to screen mothers for clinically relevant symptoms of a major depression ([Cox, 2019](#)) and the German validation study of [Bergant et al. \(1998\)](#) suggested an EPDS score of  $> 9.5$  to screen for mild depressive symptoms and a cut-off  $> 12.5$  for the diagnosis of a major depressive disorder. The German version of the EPDS revealed good split-half reliability of .82 and good internal validity of Cronbach's  $\alpha = .81$  ([Bergant et al., 1998](#)). In the current study, EPDS scores were available for  $n = 57$  mothers (two mothers did not answer item 8) and mothers had mean EPDS scores of  $M = 6.11$  ( $SD = 3.89$ , range 0–18). The analyses of interest were again tested incorporating the EPDS as an additional factor. As the

**Table 2**

Description of codes used for maternal, infant and dyadic behavior.

Code	Description
Infant social positive engagement <sup>a</sup>	Infants look at their caregiver and exhibit overall positive vocalizations, expressions and behavior. These include smiling, cooing, and laughing.
Infant social monitor <sup>a</sup>	Infants look at the caregiver, however, do not exhibit explicit positive behaviors. Infants have a neutral or interested facial expression.
Infant protesting behavior <sup>a</sup>	Infants are protesting and exhibit overall negative vocalizations, expressions and behavior. These include crying or fussing, as well as overall signs of anger and frustration. Infants are overall active, expressed by arching the back, kicking arms against the chair, trying to escape or to get picked up, as well as pushing and pulling away from caregivers.
Infant self-comforting behavior <sup>a</sup>	<i>Oral:</i> Infant stimulate themselves by sucking on their body, objects, or the caregiver's hand or fingers. There needs to be skin contact between infant's mouths and the body, objects, or caregivers. For sucking on their own body or objects, the behavior needs to be initiated by infants, for sucking on caregiver's hands or fingers, there is no rule for coding in terms of initiation. <i>Self-clasp:</i> There is contact between the hands and/or fingers of infants.
Infant positive affect <sup>b</sup>	This code is the sum of <i>infant social positive engagement</i> and <i>infant social monitor</i> .
Maternal positive affect <sup>b</sup>	This code is the sum of <i>maternal social positive engagement</i> and <i>social monitor/ positive vocalizations</i> (cf. Müller et al., 2022). <i>Maternal social positive engagement</i> is characterized by overall positive expressions, vocalizations and behavior. The mother laughs or smiles, expresses play faces, and talks to the infant in a positive manner or uses infant-directed speech. <i>Maternal social monitor/ positive vocalizations</i> is characterized by more neutral expressions and behavior, however, vocalizations are positive, i.e., mothers use infant-directed speech or make positive sounds like kissing or clicking. Her facial expression is neutral or interested. The mother is overall focused on her infant's activities.
Match <sup>b</sup>	The total amount of time mothers and infants display <i>social positive engagement</i> and/or <i>social monitor</i> (for mothers: <i>social monitor/ positive vocalizations</i> , see descriptions above) at the same time.
Interactive reparation <sup>b</sup>	The latency from the beginning of the reunion phase to the first onset of a matching state ( <i>match</i> ). This code indicates how long it takes for the dyad to restore the interaction and changing into a matching state after the disruption due to the maternal still-face. This code is given in seconds.
Dyadic eye-contact <sup>b</sup>	The mother and infant are looking at each other's face.

**Note:** All codes refer to the description in Infant and Caregiver Engagement Phases Revised (ICEP-R) Heidelberg Version by Reck et al. (2009). <sup>a</sup> These codes were used to test changes in infant affect and behavior during maternal unresponsiveness and after re-engagement. <sup>b</sup> These codes were used to test the association between SCC and HCC with maternal affect, infant affect and dyadic behavior during the last episode of the FFSF.

incorporation of the EPDS did not change the initial findings, the results can be seen in the [supplementary material](#) (see [Tables S5-S10](#)).

## 2.3. Procedure

Mother-infant-dyads came to the laboratory and were informed about the overall study procedure prior to obtaining the written informed consent. They gave the first saliva sample before starting the video observation. Mothers and infants were seated in front of each other and infants were fastened in a baby chair that was slightly elevated so that mothers and infants were approximately on eye level. Two cameras recorded maternal and infant behavior simultaneously. The examiner was in the room behind a black curtain throughout the whole procedure and preserved silent during the observation. Mothers and

infants were shielded by two partition walls during the observation. Prior to the FFSF, mothers and infants engaged in a 5-minute free play to familiarize with the setting, where mothers were instructed to engage with their infants as normally and naturally as possible. They were allowed to touch their infant, however, not to lift them out of the seat. Mothers were given an acoustic signal (i.e., a ringing bell) to indicate the start and end of the 5-minute period. Afterwards, the examiner briefly repeated the explanation of the FFSF to make sure the mother knew how to act during the procedure. The standardized FFSF (Tronick et al., 1978) consisted of three 120-seconds-periods: a play, still-face, and a reunion phase. In the play phase, mothers should continue the normal interaction with their infant. Following an acoustic signal, the mothers were instructed to turn their head to the side, to adopt a neutral (still-) face, and shift the head back towards the infant, however, looking at a point slightly above the infant's head. They were then instructed to cease all touching and to respond to none of the infants' signals by maintaining their neutral (still-) face for the following 120 seconds. After a further signal, mothers were allowed to resume the normal interaction with their infant. During the whole procedure, mothers were not allowed to lift their infant out of the seat nor to use toys. If the mother did not follow the protocol, the examiner gave a brief instruction how to act (e.g., to remain a neutral face or to engage with the infant). The examiner shortened the phase if the infant cried for 15 seconds non-stop. In the current study, play, still-face, and reunion phase had mean durations (with standard deviations in parentheses) in seconds of 117.45 (10.94), 119.48 (11.13), and 117.99 (9.57) respectively. After completion of the FFSF, mothers and infants salivated the second saliva sample. Then the mother was handed out the questionnaires. She gave information about her and the infants' social demographics (i.e., age, nationality, marital status, educational levels, infants' gestational age at birth, infant sex assigned at birth, birth weight and height), about her pregnancy (i.e., mode of delivery) and birth experience, and she completed standardized questionnaires, among others (that are not included in the current study) the EPDS. After approximately 25 minutes, the last saliva sample was gathered from both mother and infant. Lastly, the examiner cut off the hair strand of the mother. After completion, mothers received the travel refund and a child's rattle as a gift for the infant.

## 2.4. Statistical analysis

An a priori power analysis was conducted using G\*Power (version number 3.1.9.6) to determine the minimum sample size required to achieve a medium effect size of  $f^2 = .25$  in the larger study based on the study results of Tarullo et al. (2017), who investigated the association of HCC with maternal and infant behavior during a free play. To achieve 80 % power and alpha-levels set to .05, the minimum sample size for a Pearson correlation was  $N = 97$ . For the current study, a post-hoc power-analysis was run to investigate the achieved power for the current subsample ( $N = 59$ ). Looking at block-wise hierarchical multiple linear regression models (fixed model,  $R^2$  increase) that reached statistical significance and alpha-levels set to .05, the achieved power in the current study ranged between .79 and .84 (depending on the criterion).

All analyses were performed using the statistical software SPSS (version number 28.0.1.0). All alpha-levels were set to .05. We tested for the assumed still-face effect of infants during the FFSF to check for infant's reaction towards maternal unresponsiveness, using repeated measures ANOVA with the within-subjects factor *phase* (play vs. still-face vs. reunion) and post-hoc Bonferroni-adjusted pair-wise comparisons on the relative duration of *infant social positive engagement*, *infant protesting behavior* and *infant self-comforting behavior* (oral and self-clasp) during the respective phase. A decrease of *infant social positive engagement* as well as an increase of *infant protesting behavior* and *infant self-comforting* (oral and hand-grasping) behavior from play to still-face, and an increase of *infant social positive engagement* and a decrease of *infant protesting behavior* from still-face to reunion phase was assumed.

To test our hypotheses that maternal SCC and HCC relate to less *infant positive affect*, higher *infant protesting behavior*, less *maternal positive affect*, less *match*, less *interactive reparation*, and less *dyadic eye-contact*, different block-wise hierarchical multiple linear regression models were performed. Prior to the regression models, Spearman correlations were conducted to test for the association between the study variables. For all models, in a first step the possible confounding factors *maternal age*, *parity* (dummy-coded as '0 = primiparous', '1 = multiparous'), *infant gestational age* and *sex assigned at birth* (dummy coded as '0 = female', '1 = male'), were included to control for possible influences. As the sample was relatively homogeneous concerning (relatively high) educational levels (approximately 83 % of women with a university degree or higher, see Table 1), this variable was not included. Next, the additional variables HCC and SCC were added in a second model. As HCC and SCC were both not normally distributed, log-transformations with base 10 were performed prior to data analysis for HCC and the AUC<sub>G</sub> of SCC. Additional analyses were conducted to test the same hierarchical regression models as noted, however, incorporating time of day of saliva collection and maternal depressive symptoms obtained with the EPDS as additional factors to control for in the second model. The results of these analyses can be found in the [supplementary material](#) as the initial results were not changed due to the addition of these two factors (see Table S5-11).

All effects were interpreted according to Cohen (1988). For  $\eta_p^2$  this means  $\eta_p^2 = .01$  is interpreted as a small effect,  $\eta_p^2 = .06$  as a medium effect, and  $\eta_p^2 = .14$  as a large effect. Correlations were interpreted as  $r = .10$  as a small effect,  $r = .50$  as a medium effect, and  $r = .50$  as a large effect. Effects of  $R^2 = .02$  are interpreted as small,  $R^2 = .13$  as medium, and  $R^2 = .26$  as large effects.

Because of the explorative nature of our analyses, no adjustment for multiple testing has been conducted. Therefore, all results have to be interpreted as preliminary.

### 3. Results

#### 3.1. Effect of maternal still-face on infant behavior

First, the assumed still-face effect was investigated. Means and standard deviations of coded behavior can be seen in [supplemental material S 1](#). The repeated measures ANOVA for *infant social positive engagement* was significant,  $F(2, 116) = 8.16, p < .001, \eta_p^2 = .12$ . Bonferroni-corrected post-hoc tests revealed significant differences in the relative duration between play and still-face ( $M_{Diff} = 5.50, 95\% \text{ CI } [1.74, 9.25]$ ) as well as between play and reunion ( $M_{Diff} = 3.47, 95\% \text{ CI } [0.14, 6.80]$ ), however, no significant difference between still-face and reunion ( $M_{Diff} = -2.03, 95\% \text{ CI } [-5.08, 1.03]$ ). *Infant social positive engagement* decreased from play to still-face, and increased from still-face to reunion, although, infants displayed less *infant social positive engagement* during the reunion compared to the play phase. There was a significant main effect for *infant protesting behavior*,  $F(2, 116) = 27.97, p < .001, \eta_p^2 = .33$ , with significant post-hoc differences between play and still-face ( $M_{Diff} = -22.45, 95\% \text{ CI } [-34.08, -10.83]$ ), play and reunion ( $M_{Diff} = -33.23, 95\% \text{ CI } [-45.31, -21.14]$ ), and between still-face and reunion ( $M_{Diff} = -10.77, 95\% \text{ CI } [-20.45, -1.10]$ ). For *infant self-comfort (oral)*, no significant main effect could be found,  $F(2, 116) = 0.32, p = .728, \eta_p^2 = .01$ , although infants displayed descriptively more oral self-comforting behavior in the still-face phase compared to play and reunion. For *infant self-comfort (self-clasp)*, Greenhouse-Geisser correction was used to adjust for violations of sphericity. A significant main effect emerged,  $F(1.21, 70.42) = 13.82, p < .001, \eta_p^2 = .19$ , with significant differences between play and still-face ( $M_{Diff} = -9.93, 95\% \text{ CI } [-16.94, -2.92]$ ), as well as between still-face and reunion ( $M_{Diff} = 11.19, 95\% \text{ CI } [4.61, 17.76]$ ), however not for play and reunion ( $M_{Diff} = 1.26, 95\% \text{ CI } [-1.33, 3.84]$ ).

#### 3.2. Associations between maternal SCC, maternal HCC, maternal and infant affect and dyadic behavior

Results of the Spearman correlations are presented in the [supplementary material](#) (Table S 2). Regarding the block-wise hierarchical regression models, none of the first models were significant and none of the controlling factors significantly related to the criteria of interest (i.e., *infant protesting behavior*, *maternal positive affect*, *match*, *interactive reparation*, or *dyadic gaze*), except for *infant positive affect* that related to lower *infant gestational age* (see Table 3). Tables with model estimates and regression coefficients are only reported for models that could significantly explain more variance compared to the first model. The remaining tables can be found in the [supplementary material](#) (Tables S 3, S 4).

In the first block-wise hierarchical regression model, we tested for *infant positive affect*. The two factors HCC and SCC added significantly to the explanation of variance of the model with a significant change of  $R^2 = .15, F(2, 52) = 5.44, p = .007$ . *Infant positive affect* was significantly associated with lower *infant gestational age* and lower maternal SCC (see Table 3). Regarding the control variables, female infants exhibited more *infant positive affect* than male infants (see Table 3). The model coefficients and  $R^2$  can be seen in Table 3.

For *infant protesting behavior* the second model could also explain significantly more variance compared to the first model,  $F(2, 52) = 4.94, p = .011$ . The change in  $R^2$  was 15 %. However, the overall model revealed only a statistical trend, with higher maternal SCC relating to *infant protesting behavior* and male infants displaying more *infant protesting behavior* compared to girls (see Table 4 for model coefficients and  $R^2$ ).

Another block-wise hierarchical regression model tested for *maternal positive affect*. However, adding the two additional factors HCC and SCC did not explain significantly more variance,  $F(2, 52) = 0.09, p = .919$ , with a change in  $R^2 = .00$ . None of the factors were significant (see [supplementary Table S 3](#) for model coefficient and  $R^2$ ).

Testing for *match*, the second model could explain significantly more variance than the first model,  $F(2, 52) = 5.77, p = .005$ , with a change in  $R^2$  of 16 %. *Match* significantly related to lower maternal SCC (all model coefficients and  $R^2$  can be seen in Table 5). Further, dyads with lower *gestational age* and female infants also exhibited more *match* (see Table 5).

For *interactive reparation*, the second model could not explain significantly more variance compared to the first model,  $F(2, 23) = 0.04, p = .965$ . The change of  $R^2$  was 0 %. None of the factors were significant. All model coefficient and  $R^2$  can be seen in the [supplementary material](#) (Table S 4).

The last block-wise hierarchical regression model tested for *dyadic eye-contact*. Adding the factors HCC and SCC to the model added significantly to the explanation of variance,  $F(2, 52) = 3.45, p = .039$ , with a change in  $R^2 = .11$ . The second model was not significant, with lower maternal SCC relating only trend-wise to *dyadic eye-contact* (see Table 6). There was also a trend that dyads with female infants showed more dyadic eye-contact compared to dyads with male infants (see Table 6). All model coefficients and  $R^2$  can be seen in Table 6.

### 4. Discussion

The current study analyzed the association of maternal postpartum cortisol levels with mother-infant-interaction during a standardized FFSF using hair and saliva samples to measure situational and cumulated maternal cortisol levels. It could be shown that the standardized FFSF led to less positive and more negative affect after maternal non-responsiveness, aligning with previous studies (Mesman et al., 2009). Regarding the main research question on whether maternal situational and cumulated cortisol levels, indicated by SCC and HCC, relate to different aspects of mother-infant-interaction, the analyses showed a significant association of SCC with infant positive affect, infant

**Table 3**

Results of the multiple regression models for infant positive affect.

	<i>B</i>	<i>SE</i>	$\beta$	95 % CI	<i>t</i>	<i>p</i>	<i>R</i> <sup>2</sup>
<b>Model 1</b>							<b>.16 *</b>
Intercept	242.65	75.32		[91.64, 393.66]	3.22	.002 *	
Maternal age	−0.63	0.53	−0.16	[−1.70, 0.44]	−1.18	.241	
Parity	−5.57	4.60	−0.16	[−14.80, 3.66]	−1.21	.232	
Gestational age	−0.72	0.26	−0.35	[−1.24, −0.20]	−2.77	.008 **	
Infant sex	−3.98	4.38	−0.12	[−12.76, 4.80]	−0.91	.367	
<b>Model 2</b>							<b>.31 **</b>
Intercept	325.00	74.96		[174.57, 475.42]	4.34	< .001 ***	
Maternal age	−0.75	0.51	−0.19	[−1.77, 0.27]	−1.47	.147	
Parity	−2.86	4.42	−0.08	[−11.72, 6.01]	−0.65	.521	
Gestational age	−0.83	0.24	−0.41	[−1.31, −0.34]	−3.41	.001 **	
Infant sex	−7.27	4.19	0.21	[−15.68, 1.15]	−1.73	.089 <sup>†</sup>	
HCC	11.18	10.11	−0.14	[−9.10, 31.46]	1.11	.274	
SCC	−24.92	8.25	−0.37	[−41.48, −8.36]	−3.02	.004 **	

Note: *N* = 59; parity is dummy coded as '0 = primiparous' and '1 = multiparous', sex is dummy coded as '0 = female' and '1 = male', HCC = maternal hair cortisol concentration (log-transformed), SCC = maternal salivary cortisol concentration indicated as area under the curve with respect to ground (AUC<sub>G</sub>; log-transformed); \*\* *p* < .01, \* *p* < .05, <sup>†</sup> *p* < .10.

**Table 4**

Results of the multiple regression models for infant protesting behavior.

	<i>B</i>	<i>SE</i>	$\beta$	95 % CI	<i>t</i>	<i>p</i>	<i>R</i> <sup>2</sup>
<b>Model 1</b>							<b>.05</b>
Intercept	−129.48	181.88		[−494.14, 235.17]	−0.71	.480	
Maternal age	0.23	1.29	0.03	[−2.35, 2.80]	0.18	.860	
Parity	6.75	11.12	0.09	[−15.54, 29.03]	0.61	.546	
Gestational age	0.56	0.63	0.12	[−0.69, 1.81]	0.90	.375	
Infant sex	14.83	10.58	0.19	[6.37, 36.03]	1.40	.167	
<b>Model 2</b>							<b>.21<sup>†</sup></b>
Intercept	−313.18	182.48		[−679.36, 52.99]	−1.72	.092	
Maternal age	0.58	1.24	0.06	[−1.91, 3.07]	0.47	.643	
Parity	−0.01	10.76	0.00	[−21.59, 21.58]	−0.00	<i>n.s.</i>	
Gestational age	0.81	0.59	0.18	[−0.37, 2.00]	1.37	.176	
Infant sex	22.62	10.21	0.29	[−2.14, 43.11]	2.22	.031 *	
HCC	−31.56	24.60	−0.17	[−80.93, 17.81]	−1.28	.205	
SCC	55.62	20.08	0.36	[−15.32, 95.93]	2.77	.008 **	

Note: *N* = 59; parity is dummy coded as '0 = primiparous' and '1 = multiparous', sex is dummy coded as '0 = female' and '1 = male', HCC = maternal hair cortisol concentration (log-transformed), SCC = maternal salivary cortisol concentration indicated as area under the curve with respect to ground (AUC<sub>G</sub>; log-transformed); \*\* *p* < .01, <sup>†</sup> *p* < .10, *n.s.* *p* = 1.000.

**Table 5**

Results of the multiple regression models for match.

	<i>B</i>	<i>SE</i>	$\beta$	95 % CI	<i>t</i>	<i>p</i>	<i>R</i> <sup>2</sup>
<b>Model 1</b>							<b>.13</b>
Intercept	224.66	95.32		[33.55, 415.77]	2.36	.022 *	
Maternal age	−0.64	0.67	−0.13	[−1.99, 0.71]	−0.95	.345	
Parity	−8.41	5.83	−0.20	[−20.09, 3.27]	−1.44	.155	
Gestational age	−0.62	0.33	−0.25	[−1.28, 0.04]	−1.89	.064 <sup>†</sup>	
Infant sex	−7.06	5.54	−0.16	[−18.17, 4.05]	−1.27	.208	
<b>Model 2</b>							<b>.28 **</b>
Intercept	333.21	94.38		[143.83, 522.60]	3.53	< .001 ***	
Maternal age	−0.78	0.64	−0.16	[−2.06, 0.51]	−1.21	.231	
Parity	−5.03	5.56	−0.12	[−16.20, 6.13]	−0.91	.370	
Gestational age	−0.77	0.31	−0.31	[−1.38, −0.15]	−2.51	.015 *	
Infant sex	−11.25	5.28	−0.26	[−21.85, −0.66]	−2.13	.038 *	
HCC	12.85	12.72	0.13	[−12.69, 38.38]	1.01	.317	
SCC	−32.84	10.39	−0.39	[−53.69, −12.00]	−3.16	.003 **	

Note: *N* = 59; parity is dummy coded as '0 = primiparous' and '1 = multiparous', sex is dummy coded as '0 = female' and '1 = male', HCC = maternal hair cortisol concentration (log-transformed), SCC = maternal salivary cortisol concentration indicated as area under the curve with respect to ground (AUC<sub>G</sub>; log-transformed); \*\*\* *p* < .001, \*\* *p* < .01, \* *p* < .05, <sup>†</sup> *p* < .10.

protesting behavior, dyadic match, and dyadic eye-contact during the reunion phase. The variance explained could be considered as small to moderate. HCC did not significantly relate to any of the investigated aspects of the mother-infant-interaction. Additionally, the analyses revealed significant associations with infant gestational age, and infant sex assigned at birth on infant positive affect, infant protest behavior and

match. Additional analyses in the supplementary sample included two more factors, time of day and maternal depressive symptoms, that were entered as additional factors in the hierarchical regression model as both factors can potentially affect the association between SCC and HCC with different maternal, infant and dyadic behaviors. However, compared to the models presented in the main text, the additional factors time of day



**Table 6**

Results of the multiple regression models for dyadic eye-contact.

	<i>B</i>	<i>SE</i>	$\beta$	95 % CI	<i>t</i>	<i>p</i>	<i>R</i> <sup>2</sup>
<b>Model 1</b>							<b>.09</b>
Intercept	179.14	101.90		[-0.25, 3.84]	1.76	.084 <sup>†</sup>	
Maternal age	-0.59	0.72	-0.11	[-0.02, 0.01]	-0.82	.418	
Parity	-8.07	6.23	-0.18	[-0.21, 0.04]	-1.30	.201	
Gestational age	-0.44	0.35	-0.17	[-0.01, 0.00]	-1.26	.215	
Infant sex	-7.00	5.92	-0.16	[-0.19, 0.05]	-1.18	.243	
<b>Model 2</b>							<b>.19<sup>†</sup></b>
Intercept	276.15	104.78		[0.66, 4.86]	2.64	.011 *	
Maternal age	-0.65	0.71	-0.12	[-0.02, 0.01]	-0.91	.366	
Parity	-5.55	6.18	-0.12	[-0.18, 0.07]	-0.90	.373	
Gestational age	-0.57	0.34	-0.22	[-0.01, 0.00]	-1.68	.100	
Infant sex	-10.40	5.86	-0.23	[-0.22, 0.01]	-1.78	.082 <sup>†</sup>	
HCC	6.77	1.134	0.06	[-0.22, 0.35]	0.48	.634	
SCC	-29.33	11.53	-0.33	[-0.53, -0.06]	-2.54	.014 **	

Note: *N* = 59; parity is dummy coded as '0 = primiparous' and '1 = multiparous', sex is dummy coded as '0 = female' and '1 = male', HCC = maternal hair cortisol concentration, SCC = maternal salivary cortisol concentration indicated as area under the curve with respect to ground (AUC<sub>G</sub>); \*\* *p* < .01, \* *p* < .05, <sup>†</sup> *p* < .10.

and maternal depressive symptoms were not significantly associated with the investigated aspects of the mother-infant-interaction. The current results should be considered as preliminary, especially, as the effect of situational cortisol possibly needs to be investigated under more standardized conditions. However, the results give valuable insights into the complex interplay between maternal cortisol and mother-infant-interaction and possible endpoints for future studies.

To investigate the association of maternal cortisol with the mother-infant-interaction, the current study used SCC as a marker for situational and HCC as a retrospective marker for cumulated maternal cortisol levels. We found a significant negative association between maternal SCC and infant positive affect, dyadic match, and dyadic eye-contact, as well as a positive association with infant protest during the reunion phase of the FFSF, while maternal positive affect and interactive reparation were unrelated to SCC. Mueller et al. (2021) demonstrated that offering an experimental stressor to the mother prior to the FFSF led to increased infant's distress during the FFSF and that observations had to be terminated earlier, although the authors did not control for an increase in maternal cortisol levels. Cortisol elevation following a stressor can demand cognitive resources that potentially impair maternal sensitivity, as indicated by studies that found effects of cortisol on cognitive flexibility (Goldfarb et al., 2017; Plessow et al., 2011) and on a shift from sensitive to more habitual behaviors (Schwabe and Wolf, 2013). This can possibly also affect the mother-infant-interaction, although this was not yet addressed in a corresponding study design. Thompson and Trevathan (2008) did not facilitate an experimental stressor, however, found that cortisol increase could be associated with less synchronized behavior between mothers and their 3-month-old infants during a free-play. This could reflect the aforementioned association between cortisol and maternal capabilities to engage in sensitive caregiving. The current study did not incorporate a standardized stress paradigm. Therefore, it is not clear if and what could have led to an increase in SCC in some mothers, but not in others. The arrival to the laboratory in the current study is one factor that may have facilitated a cortisol response in some mothers more than others resulting in heightened SCC at the beginning of the experiment. An increase in cortisol may have resulted in impaired maternal capabilities to engage in dyadic behavior, which was reflected by less dyadic match and eye-contact in dyads with higher maternal SCC. Following the mutual regulation model (DiCorcia and Tronick, 2011; Gianino and Tronick, 1988), a mother with higher cortisol levels possibly had more difficulties to repair the interaction with her infant and to change back from a mismatching state to a matching state. This could be associated with cognitive and behavioral impairments due to momentary elevated cortisol levels (Goldfarb et al., 2017; Plessow et al., 2011; Schwabe and Wolf, 2013). A mere maternal positive affect, which was unrelated to SCC in the current study, possibly was insufficient to co-regulate infants

after maternal unresponsiveness. Further, infants of mothers with higher SCC exhibited more negative and less positive affect during the reunion, possibly as an infant's response towards less dyadic coordination, which is comparable to the findings of Mueller et al. (2021). A reciprocity between maternal elevated cortisol levels and infant affect during interactions, however, cannot be ruled out either. Previous research suggests that infants born to mothers with increased cortisol levels during pregnancy are in general higher in irritability (Takegata et al., 2021), which may contribute to less favorable dyadic and positive interaction patterns. Therefore, maternal cortisol responses could be generally higher due to a more difficult temperament of infants and vice versa regardless of the study setting. Other studies reported associations between averaged cortisol levels during a mother-infant-observation with maternal sensitivity (Finegood et al., 2016), or intrusive maternal behavior (Mills-Koonce et al., 2009), which is surprising as maternal positive affect and interactive reparation seemed unaffected by SCC in the current study. Otherwise, studies also linked higher cortisol levels measured from saliva to more adequate caregiving behavior and a higher alertness towards infant cues (Fleming et al., 1997). Shortly after birth, cortisol may lead to more caregiving behavior and higher responsivity towards infant cues (Bos, 2017), whereas later on, elevated levels of cortisol can be associated with less optimal caregiving, maternal sensitivity and responsiveness, and more negative interaction patterns (Mills-Koonce et al., 2009). Lastly, some studies also found no such effects of cortisol on maternal caregiving behavior (Bos et al., 2018). That the current study found no association between SCC and maternal behavior may depend on the study design itself. The laboratory observation may facilitate more positive behaviors in mothers (Belsky, 1980; Zegib et al., 1975) as indicated by high amounts of maternal smiling and positive vocalizations during more than 90 % of the time during the play and reunion phase of the current study. Studies found that mothers engage in a more positive (socially desirable) way in laboratory observations compared to more naturalistic at home observations (Belsky, 1980), and that being uninformed about being observed during a mother-infant-interaction led to less positive behavior and less adjusting of infant's behavior compared to being informed (Zegib et al., 1975). However, at home observations potentially lack standardization and comparability, and leaving the women uninformed about being observed faces ethical and privacy policy considerations. Further, Mesman et al. (2013) found comparable results in a FFSF conducted at families homes, suggesting reliable still-face effects regardless of the setting.

In the current study, we were also interested in the association between more long-time cortisol levels and the mother-infant-interaction using HCC, a marker for cumulated cortisol levels. No significant association with maternal, infant, or dyadic behavior emerged. This contradicts the results of Tarullo et al. (2017) that higher HCC related to

more negative and fewer positive behaviors in mother-infant-dyads during a free play interaction. Other studies obtained maternal HCC during pregnancy. They found that elevated HCC in the third trimester could be associated with more intrusive maternal behavior as well as maternal withdrawal during the FFSF paradigm, however, only when mothers additionally exhibited depressive symptoms at 4 months postpartum (Khoury et al., 2020). Nystrom-Hansen et al. (2019) assessed maternal HCC during the third trimester and again 4 months postpartum and found a significant association between maternal HCC and mental illnesses (e.g., depression, bipolar disorder, schizophrenia) as well as disrupted mother-infant-interaction 4 months postpartum. The authors further found HCC (during the third trimester and 4 months postpartum) to mediate the association between maternal mental illness and disrupted mother-infant-interaction (Nystrom-Hansen et al., 2019). In the current study, maternal mental illness was not considered as an associated factor. Conversely, long-term mental illnesses can also down-regulate the HPA axis and lower cortisol levels (Pochigaeva et al., 2017). Our sample had relatively low maternal HCC compared to other studies (Kirschbaum et al., 2009), possibly due to only mild stressors during the past months, a chronic downregulation of the HPA axis, or hormonal changes associated with pregnancy and birth, which cannot further be assessed. An additional control for mental illnesses or life events is indicated, however, self-reported measures for mental illnesses and HCC seem to not always be correlated (Braig et al., 2016). In the current study, maternal depressive symptoms assessed by the EPDS did not change the overall results. Further, a preceded pregnancy and concomitant tremendous changes in hormonal levels potentially impair the interpretation of cortisol levels. HCC as a retrospective marker for cumulative cortisol levels reflects a certain period of time depending on the length of the observed hair strand. As infants were 4 months of age, we investigated a time frame shortly after birth which may be specifically affected by the hormonal changes due to pregnancy and birth: Hair cortisol levels of pregnant women in contrast to non-pregnant women are increased from third trimester up to 3 months postpartum (Kirschbaum et al., 2009). Galbally et al. (2019) found HCC increases during pregnancy until 3 months postpartum and then decrease from 3 months postpartum to 12 months postpartum. The current study might have captured a sensitive period with rapid changes in maternal cortisol levels due to hormonal shifts, and maternal HCC may reflect these changes rather than the presumed cumulated cortisol levels as a consequence of stress. Another time point when hormonal levels of mothers are comparable to levels of non-pregnant women may be indicated. However, more research is highly needed as current results are scarce and only few studies investigated HCC during the postpartum period.

The current study considered maternal and infant characteristics that were described to be important while investigating associations between maternal cortisol levels and the mother-infant-interaction. However, only infant gestational age and sex assigned at birth significantly related to infant affect and dyadic interaction. Infant gestational age was negatively associated with infant positive engagement and dyadic match during reunion, challenging a broad body of literature suggesting that lower gestational age, often reflected by preterm infants, relates to less positive and synchronized parent-infant-interaction (for an overview see e.g., Bilgin and Wolke, 2015). As the current study investigated only full-term infants (i.e., born  $\geq$  37th week of gestation) applicability of these results is unclear. Research on the effects of gestational age within the normal range is scarce, suggesting the need for more comprehensive studies on gestational age within this range. Our analysis revealed that female infants displayed more positive affect, less protest behavior, as well as more dyadic interaction during the reunion phase. Findings considering sex differences in infant affect during the FFSF are heterogeneous (Alexander and Wilcox, 2012), however, there are studies reporting that infant boys seem to be more prone to the maternal still-face (Weinberg et al., 1999), which can also be inferred from our study results. As sex differences in infant behavior are evident quite

early after birth and a reinforcement of such gender differences by socialization factors is likely (Alexander and Wilcox, 2012), infant sex assigned at birth seems to be an important variable to include in studies investigating mother-infant-interactions.

The current study has some methodological considerations and limitations. HCC can be affected by several other factors, e.g., socio-economic status, traumatic events, UV radiation, chemical hair treatment or washing (Greff et al., 2019; Stalder et al., 2017), that may impede study results. Further, cortisol measured in hair or saliva is directly affected by sampling time, physical activity, food intake, sleep, or smoking (Kirschbaum and Hellhammer, 2008; Stalder et al., 2017), for which the current study did not account and should therefore be interpreted as preliminary. However, as can be seen by the additional analyses in the [supplementary material](#), the addition of time of sampling of saliva samples as a factor did not change the overall results. The findings of this study are correlational in nature, and thus, caution must be exercised in drawing causal inferences. To improve the current correlative study design, future studies could integrate standardized stress paradigms (see Mueller et al., 2021) and endocrine markers that reflect the human stress response to test for effects on the mother-infant-interaction. Next, although the laboratory assessment allows for standardization, it may not capture the mother-infant-interaction in a naturalistic way (Belsky, 1980; Zegib et al., 1975) as noted earlier discussing the results on SCC. As our sample was quite homogenous and high in educational levels, a more diverse sample is indicated. The current study only investigated mothers, however, for infant development other caregivers like fathers seem just as important (Jansen et al., 2024) and should be evenly investigated in future studies.

Lastly, we want to emphasize some strengths of the current study. To the best of our knowledge, this is one of the first studies investigating the effects of maternal cortisol levels and the mother-infant-interaction during a standardized FFSF using two endocrine parameters HCC and SCC, alongside the study by Tarullo et al. (2017) who, however, used a free play observation. Our study adds valuable information about the impact of maternal cortisol during the early postpartum period for the mother-infant-interaction while controlling for several mother and infant characteristics. Further, to analyze the mother-infant-interaction, we have used a microanalytical approach. This allows to capture more fine-grained patterns of interaction compared to macro-analytical approaches (Lotzin et al., 2015).

## 5. Conclusion

This is one of the first studies examining the association between maternal cortisol and the mother-infant-interaction by employing maternal HCC and SCC in one study under consideration of potentially relevant maternal and infant characteristics. Model estimates have to be considered as low to moderate, but the results indicated that maternal SCC is related to infant and dyadic behavior, while HCC did not relate to any facet of the mother-infant-interaction. Interestingly, maternal positive interaction did not reveal any association with the considered variables, either because the behavior is rather unaffected by maternal cortisol, or it was distorted by the laboratory setting. Infant gestational age and sex assigned at birth emerged as significant factors relating to mother-infant-interaction, suggesting the need for their inclusion as control variables in future studies. Given the exploratory approach of our study, our results need to be validated in future research that allows for more causal interpretation.

Investigating the effects of maternal cortisol during the postpartum period on the mother-infant-interaction is of high significance, as dyads that cannot effectively repair their interaction after a disruption could face risks: infants potentially fail to learn adequate self-regulation strategies (DiCorcia and Tronick, 2011), and caregivers potentially build negative expectations when interacting with their infants (Seymour et al., 2015), possibly leading to long-term psychosocial consequences

and emotion-regulation problems (Deans, 2020). Therefore, it is crucial that future research, building on the preliminary findings presented here, be conducted to identify caregivers at risk for developing problematic parent-infant interactions during the early stages of infant development.

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## CRediT authorship contribution statement

**Nora K. Schaal:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Martin Heil:** Writing – review & editing, Methodology. **Oliver T. Wolf:** Writing – review & editing, Methodology. **Juliane Tautz:** Writing – review & editing, Methodology. **Nora Nonnenmacher:** Writing – review & editing, Methodology. **Sabine Seehagen:** Writing – review & editing, Methodology. **Luisa Ernten:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that there is no competing interest.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2024.107266](https://doi.org/10.1016/j.psyneuen.2024.107266).

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