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Cortisol reactivity and negative affect among preterm infants at 12 months during a mother-infant interaction task

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A R T I C L E I N F O

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A B S T R A C T

The purpose of this study was to investigate correlates of preterm (PT) infant's cortisol reactivity and the association to infant negative affect, during a mother-infant interaction procedure. Participants included 48 infants born prematurely (gestational age < 37 weeks) and their mothers, assessed when infants were 12 months old corrected for prematurity. The examined variables comprised both neonatal and environmental dimensions including maternal interactive behavior. Infant negative affect and maternal interactive behavior were assessed with a standardized mother-infant interaction task. A baseline infant saliva sample was collected before the interaction began, and a second sample after the interaction episodes ended. Results revealed that decrease of infant's cortisol concentration was significantly associated with the exposure to more sensitive, and less intrusive maternal behaviors. However, once controlled for neonatal risk, family SES and maternal psychological distress, the associations were rendered non-significant. Although the association between cortisol reactivity and negative affect trended toward significance, maternal intrusiveness was the only significant predictor of observed infant negative affect. Findings suggest the importance of primary relational experiences on PT infants' early regulatory competencies.

1. Introduction

Preterm (PT) infants are at elevated risk for a myriad of developmental problems with long term implications that might persist up to adulthood (Aarnoudse-Moens, Weisglas-Kuperus, van Goudoever, & Oosterlaan, 2009; Allotey et al., 2018; Arpi & Ferrari, 2013; Favrais & Saliba, 2019; Pascal et al., 2018; Wolke, Johnson, & Mendonça, 2019). Research conducted over the last years has provided a growing body of evidence on the presence of neuroendocrine dysfunctions—i.e., altered (re)activity of the hypothalamic-pituitary-adrenal (HPA) axis—, resulting in PT infant's impaired ability to regulate their physiological stress responses (for a review, see Finken et al., 2016, 2017; Valeri, Holsti, & Linhares, 2015). Grunau et al. (2007) have first described a developmental trajectory of cortisol levels in PT infants, suggesting the existence of a re-setting of neuroendocrine systems over time. It was found that long-term programming of the HPA axis in PT was characterized by a shift from dampened basal cortisol concentration at 3 months to

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significantly high concentrations at 8 and 18 months compared with full-term children (Grunau, et al., 2007). Accordingly, subsequent investigation with 3-month-old PT infants found blunted salivary cortisol responses to socio-emotional stress (Provenzi et al., 2016), whereas studies with older infants supported the presence of higher basal cortisol levels and enhanced cortisol responsivity patterns in response to stress eliciting situations among 18-month-old preterm infants (Brummelte et al., 2011).

The above evidence is consistent with a downregulation in PT newborns due to relative adrenal insufficiency (Fernandez & Watterberg, 2009), with a possible change to an upregulation pattern later on in their developmental course. Furthermore, there may be long-lasting effects of HPA axis dysregulation with the presence of altered cortisol levels later in childhood (Buske-Kirschbaum et al., 2007; Quesada, Tristão, Pratesi, & Wolf, 2014) and adulthood (Kajantie et al., 2002; Winchester, Sullivan, Roberts, & Granger, 2016).

There is a good deal of evidence suggesting that the mechanisms underlying this dysregulation are associated with a constellation of birth-related stressors (Brummelte et al., 2015; Newnham, Inder, & Milgrom, 2009; Sullivan, Winchester, Bryce, & Granger, 2017; Valeri et al., 2015). Indeed, being born PT is associated with exposure to an environment that differs markedly from that of children born healthy and full-term. The premature infant faces the constraints of its fragile and immature physiological condition along with the harshness of the NICU environment and procedures. During neonatal care, preterm infants may undergo numerous invasive and painful procedures that have been associated with neurodevelopmental impairments across infancy (Vinall et al., 2014). Not least important, maternal separation during NICU stay constitutes an important concurrent social stress source (Grunau, 2013). Studies have proposed that exposition to such stressful and potentially painful experiences during a critical phase of rapid brain development may have the potential to change brain microstructure and function, and alter permanently the configuration and programming of stress systems (Anand & Scalzo, 2000; Brummelte et al., 2015; Grunau, 2013; Matthews, 2002). In a review, Mörelius, He, and Shorey (2016), showed that painful interventions could provoke a significant elevation in PT infant's salivary cortisol, nonetheless, the impact of other routinely NICU procedures, such as, separation from the parental figures, needs further investigation.

Therefore, research has sought to determine the factors associated with PT infant's HPA axis dysregulation, with a particular focus on neonatal conditions and medical procedures. Among those factors, studies have consistently shown the influence of low gestational age and low birth weight (Brummelte et al. 2011; Grunau et al., 2007; Scott & Watterberg, 1995) and of painful procedures that are frequently performed during neonatal intensive care (NICU) (de Graaf et al., 2014; Grunau, Weinberg, & Whitfield, 2004; Grunau et al., 2005, 2013; Holsti, Weinberg, Whitfield, & Grunau, 2007). The lower the gestation, the more often preterm infants experience interventions and thus prematurity or birth weight is often intertwined with stress-induced interventions such as mechanical ventilation (Grunau et al., 2007; Gunes, Koklu, Ozturk, Koklu, & Cetin, 2006).

In contrast to the research on neonatal infants or treatment factors related to HPA-axis activity in the infant, there is a paucity of studies that investigated environmental factors beyond the neonatal period that may contribute to modulating PT infants' stress systems. Research with children born full-term (FT) revealed that HPA axis functioning is highly influenced by early adverse environmental experiences, including maternal psychological distress (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Braren, Perry, Ursache, & Blair, 2019; Essex, Klein, Cho, & Kalin, 2002; Ulmer-Yaniv, Djalovski, Priel, Zagoory-Sharon, & Feldman, 2018). Along with that, it is extensively described that the circumstances involved in PT birth are highly stressful and potentially traumatic for parents, exposing them to a higher risk of showing psychological problems (Forcada-Guex, Borghini,Pierrehumbert, Ansermet, & Muller-Nix, 2011; Yaari, Treyvaud, Lee, Doyle, & Anderson, 2019), which in turn may harm children's neuroendocrine functioning.

Besides, from a more proximal level, research has highlighted the role of primary attachment relationships and maternal behavior as crucial social stress buffers during the first years of life (Boyce & Ellis, 2005; Gunnar, 1998; Hostinar, Sullivan, & Gunnar, 2014). Indeed, the stress-buffering effect of social relationships is widely documented across species, with the role of the primary caregiver, generally the mother, as the most powerful social buffer (for reviews see Gunnar & Quevedo, 2007; Hostinar et al., 2014). Studies with FT infants have shown that sensitive caregiving is associated with more optimal stress responses. In particular, more sensitive and less intrusive maternal behavior has been reported to be associated with lower and more regulated cortisol activity, functioning as an external regulator of infant neuroendocrine systems activity (e.g., Albers, Riksen-Walraven, Sweep, & de Weerth, 2008; Bosquet Enlow et al., 2014; Martinez-Torteya et al., 2014; Tarullo, John, & Meyer, 2017). However, this also means that if the primary caregiver is unable to provide adequate caretaking, maternal behavior may instead become a major source of stress for children (Gunnar & Quevedo, 2007).

It has been suggested that infants born prematurely may be especially sensitive or vulnerable to the effects of the environmental context such as maternal care (Forcada-Guex, Pierrehumbert, Borghini, Moessinger, & MuUer-Nix, 2006; Gueron-Sela, Atzaba-Poria, Meiri, & Marks, 2015; Jaekel, Pluess, Belsky, & Wolke, 2015; Poehlmann, Schwichtenberg, Shlafer, et al., 2011). Thus, maternal caretaking may be a central factor to investigate as being associated with PT infant's stress-responsive biological systems activity. For instance, Brummelte et al. (2011) compared the patterns of cortisol responsiveness to a cognitive task in PT and FT children and investigated the relation with maternal interactive behaviors. Results revealed that higher cortisol levels in PT infants, but not in FT counterparts, were associated with less optimal mother interactive behaviors, suggesting PT infants' greater sensitivity to maternal care vis-a-vis cortisol regulation. Besides, recent findings suggest that seems to be especially challenging for dyads of very preterm infants and their mothers to engage in a dynamic co-regulatory process of the HPA axis, resulting in difficulties in reciprocally adapting to stressful conditions. Differently from dyads of FT infants, preterm infants and their mothers fail to coupled their biological rhythms and to jointly regulate their internal states (Provenzi et al., 2019).

1.1. Behavioral correlates of cortisol dysregulation

Another issue yet to explore is whether PT infant's physiological reactivity correlates to behavioral reactivity. Studies with full-term children have examined this relationship but reported mixed findings. For instance, Lewis and Ramsay (2005) found that cortisol responses were positively correlated with negative affect reactivity, in terms of sadness. Similarly, Donzella, Gunnar, Krueger, and Alwin (2000) found a positive correlation between cortisol reactivity and tense/angry affect during a stressor paradigm. However, behavioral and physiological distress are not always correlated (Jansen, Beijers, Riksen-Walraven, & de Weerth, 2010). Infants might experience a decrease in cortisol responses but still, exhibit expressions of distress. The opposite is also possible to occur (Hostinar et al., 2014). It may be the case that developmental changes may affect the associations between the two dimensions. For instance, Gunnar et al. (1996) found statistically significant correlations between cortisol and behavior at 2, 4, and 6 months of age; however, by 15 months, none of the cortisol-behavior correlations were significant, suggesting that the pattern of such correlations may change with time.

Surprisingly, the associations between physiological and behavioral reactivity are still unexplored among infants born preterm. This is surprising considering that has already been shown that PT infants may be at higher risk of showing regulatory difficulties physiologically and behaviorally (Bilgin & Wolke, 2016; Clark, Woodward, Horwood, & Moor, 2008; Feldman, 2009) but the two dimensions have rarely been studied together. There is some evidence documenting PT infant's higher levels of behavioral reactivity and negative affectivity temperament, with important long-term consequences to their behavioral and emotional development, and later psychological well-being (Hughes, Shults, McGrath, & Medoff-Cooper, 2002; Klein, Gaspardo, Martinez, Grunau, & Linhares, 2009; Leaberry, Rosen, Slaughter, Reese, & Fogleman, 2019; Pesonen et al., 2009).

Although the association between cortisol and behavioral reactivity in infants born preterm is still unexplored, some notable exceptions produced mixed results. For instance, Erickson, Maclean, Qualls, and Lowe (2013) used a modified Still-Face (ST) protocol to assess infant positive and negative affect and their mothers' interactive behaviors, in a cohort of 6–8-month-old infants born preterm. Infant saliva samples were collected, before testing began and 20 and 30 min after the first ST episode. They found that cortisol responses were positively correlated to both negative and positive affective responses, suggesting a possible asynchrony between behavioral and physiological responses to a stressful situation. In another study, Habersaat et al. (2013) compared emotional and cortisol responses in very preterm and full-term infants at six months of age and found no evidence of an association between neuroendocrine and emotional regulations.

In summary, there is a paucity of studies of HPA-axis regulation and negative reactivity in PT populations, and the limited findings in PT but also FT children have produced inconclusive results. Additionally, whether PT may be more sensitive or vulnerable to the effects of certain stressors such as less than optimal caretaking, needs further investigation.

Table 1
Descriptive statistics of child and family characteristics.

Variable		
Child Birth Characteristics		
	M (*SD*; range) gestational age (weeks)	33.10 (2.59; 27–36)
	M (*SD*; range) birth weight (grams)	1956.79 (593.60; 706–1959.79)
	% Male	56.3
	% Twin	27.1
	% Diagnosis of apnea	20.8
	% Respiratory distress	29.2
	% Bronchopulmonary dysplasia	10.4
	% Gastroesophageal reflux	4.2
	% Supplementary oxygen at discharge	4.2
	% 5-min Apgar score $\leq$ 6	2.1
	% ventilation during NICU stay	43.8
	% NICU stay $\geq$ 30 days	29.2
Family socio-demographic characteristics		
	M (SD) Family monthly income (euros)	1510.43 (686.35)
	M (SD) Number of household members	3.79 (1.19)
	M (*SD*) Maternal age (years)	34.44 (4.97)
	Maternal education	
	%Less than nine years of education	27.1
	Paternal education	
	% Less than nine years of education	25
	%Maternal unemployment	27.1
	%Paternal unemployment	6.3
Maternal psychological distress		
	M (*SD*) Psychological symptoms (BSI)	1.37 (0.28; 1 – 2.27)
	M (*SD*) Perceived stress	35.95 (24.97;0.00 – 98.00)

Note. NICU = Neonatal Intensive Care Unit; BSI = Brief Symptom Inventory.

1.2. The current study

The aims of the present study were two-fold: (1) to examine the correlations between both neonatal and environmental factors, including maternal sensitivity and cooperation, and preterm infant's cortisol reactivity during a mother-infant interaction procedure; and (2) to investigate the association between cortisol and behavioral reactivity, assessed in terms of infants' negative affect, during a mother-infant interaction task. Taking into consideration the literature, we hypothesized that (1) less sensitive and more intrusive maternal behaviors would be related to higher levels of cortisol reactivity, even after controlling for infants' neonatal risks; and that (2) higher levels of cortisol reactivity would correlate with higher expression of preterm infants' negative affect.

2. Method

2.1. Participants

Participants of the present study were drawn from a larger longitudinal study aimed to investigate the biological and environmental predictors of self-regulation and socio-cognition in preschoolers born preterm during the first 3 years post-partum (for an example of other studies within this project, see [blind review]). The larger study recruited 150 preterm-born children and their parents, from two hospitals in Northern Portugal and comprised three assessment points (i.e., when children were 12 months of corrected age (T1); 24 months of corrected age (T2), and 3 years-old of chronological age (T3). The current study comprised a sub-sample of 48 preterm infants with 12 months old (27 boys, 56.3 %) with two valid saliva samples that enabled the determination of cortisol concentration variation. The saliva collecting procedure was conducted with all 150 infants that participated at the first assessment point from the larger study, however, for most of the infants, we could not obtain a sufficient amount of saliva to proceed with cortisol concentration determination, at least at one of the sampling times (e.g., valid saliva sample at T1 but not enough saliva for analysis at T2). There were no significant differences between participants included in this study and those from whom it was not possible to determine cortisol concentration variation regarding infant gestational age, birth weight, and maternal education (all $p > .05$). All participating infants were born in Portugal during the period 1 July 2013–31 December 2015. The study only included healthy infants, according to the following criteria: gestational age < 37 weeks and absence of congenital or current neurological problems, chromosomal disorders, and/or fetal drug/alcohol exposure. Following previous recommendations (Brummelte et al., 2011), we did not exclude infants whose mothers received antenatal corticosteroids. Mothers age varied between 20 and 41 years old (M = 34.44 years, SD = 5.0). Table 1 provides detailed sociodemographic and neonatal information of participants.

2.2. Procedure

The research was approved by the Portuguese National Commission for Data Protection and by the ethical boards of the participating hospitals and the university involved in the coordination of the study. Families were first contacted and informed about the study by their pediatrician. Those who agreed to participate were then contacted by a researcher and a visit was scheduled to the hospital for data collection, when infants were 12 months of corrected age. Participation in the research was voluntary and participants received no financial compensation for taking part in the study.

All assessments were conducted in the hospital setting. The visit started with the study explanation and then written informed consent was obtained from all the mothers. Infant mental development was measured by a trained child psychologist, while mothers were asked to complete demographic forms (e.g., socioeconomic information including maternal education) and to fill out questionnaires assessing self-reported psychological distress.

The mother and infant were enrolled in a 15-minute interactive task, as described by Baptista, Silva, Marques, Martins, and Soares (2018), designed to assess caregiver's interactive behaviors. The task is structured to progressively increase the challenge for the dyad across three episodes, involving: (a) mother and infant play with developmentally appropriate toys (5 min: "Please, play with [name of the child] as you usually do, using these toys (e.g. dolls, toy phones, blocks, balls, books, puppets"); (b) mother and infant play without toys (5 min*:* "Now, could you please play with your child without any toy during the next 5 min"); and (c) mother and infant play with a "difficult-to-use" toy (5 min: "Now, please, play with [name of the child], using this shape sorting cube. This toy might be difficult for [name of the child] thus I ask you to try to teach him how to play with it during the next 5 min").

The interaction was videotaped for later coding of maternal sensitivity and cooperation, and infant negative affect. A basal infant saliva sample was collected before the interaction began (T1), and a second sample after the interaction episodes ended (T2).

2.3. Measures

2.3.1. Observed infant negative affect

Infant disposition to react negatively was assessed based on the videotaped mother-infant interactive task using the coding system for mother-child interactions developed by the National Institute of Child Health and Human Development Network (NICHD Early Child Care Research Network, 1999; Portuguese adaptation by Freixo & Baptista, 2016). The coding system included seven maternal subscales (i.e., sensitivity to distress; intrusiveness; detachment; stimulation of cognitive development; positive regard for the child; negative regard for the child; and flatness of affect) and five children sub-scales (i.e., positive affect, negative affect, activity level, sustained attention, sociability to others) coded on a 4-point Likert scale (1 = not at all characteristic, to 4 = highly characteristic). In this particular study, only the negative affect subscale was considered. The negative affect subscale assesses the extent to which the infant expresses discontentment with the situation. Measures of infant negative affect include crying, fussing, frowning, and body tension while crying. To examine interrater reliability, 31.25 % of the cases (N = 15) were double-coded by trained independent ratters that were blind to all other assessments. The inter-rater agreement was good before consensus scoring of disagreements on ratings (negative affect, ICC = 0.82).

2.3.2. Infant cortisol reactivity

Salivettes (Sarstedt, Rommelsdorf, Germany) were used to collect the infant's saliva for cortisol concentration analysis. Mothers were asked to apply a cotton dental roll into the mouth of their infants, in contact with their tongue, cheeks, and gums, long enough for the roll to become moist. According to the parent report, all infants were healthy on the test day and had not been exposed to any potentially stressful events (e.g., vaccination) in the previous days. All saliva samples were obtained during afternoon time and infants were not fed within approximately 30 min before basal cortisol collection. Saliva samples were collected before the beginning of the mother-infant interaction (T1) and after the interaction episodes ended (T2). Salivettes were kept chilled before being centrifuged at 4 °C at 3200 rpm for 10 min. Samples were then stored at − 80 °C. The determination of cortisol concentration was performed using an Enzyme-linked immunosorbent assay kit (ELISA) (IBL International GmbH, Hamburg, Germany), following the manufacturer's instructions. According to previous research (Thompson & Trevathan, 2008), cortisol data were examined for outliers, and T1 and T2 values that were ± 3 *SD* from the mean were discarded. Only one score fitted this criterion. The raw scores were transformed to log-10 scores, as they were not normally distributed. All the following statistical analyses were performed using the log 10-values. Cortisol reactivity was calculated based on the difference between T1 and T2 (ΔT1-T2). Based on previous studies, cortisol reactivity was only considered when ΔT1-T2 was 0.5 *SD* from the mean of the raw score, indicating that an effective change in cortisol level occurred (Thompson & Trevathan, 2008). Salivary cortisol at T1 ranged from.02 to 0.32 u/ml ($M = 0.08$, $SD = 0.06$), and at T2 ranged from.02 to 0.51 u/ml ($M = 0.09$, $SD = 0.09$).

2.4. Covariates

2.4.1. Infant neonatal risks

Based on the infant's Neonatal Intensive Care Unit (NICU) medical records a neonatal health risk index was calculated, as described by Poehlmann et al. (2012). Child gestational age and birth weight were standardized, reverse-scored, and combined with the standardized sum of the presence of ten neonatal medical risk factors: diagnosis of apnoea, respiratory distress, chronic lung disease, gastroesophageal reflux, multiple birth, supplementary oxygen at NICU discharge, apnoea monitor at NICU discharge, 5- min Apgar score ≤ 6, ventilation during NICU stay, and NICU stay ≥ 30 days. Higher scores represent a higher neonatal risk.

2.4.2. Maternal interactive behavior

The Ainsworth, Blehar, Waters, and Wall (1978) Sensitivity/Insensitivity and Cooperation/Intrusiveness scales were used by highly trained raters to assess the quality of maternal interactive behavior during the 15-minute videotaped interaction episodes. The Sensitivity-Insensitivity subscale reflects the mother's ability to accurately perceive infant signals and communications, interpret them correctly, and respond to them promptly and adequately. The Cooperation-Intrusiveness subscale assesses the mother's ability to respect the infant's autonomy and to follow their interests and mood rather than to break into or interrupt the infant's ongoing activity or exert direct control. Each subscale was rated on a 9-point scale, with higher scores indicating more sensitive and cooperative maternal behavior, respectively. To examine interrater reliability, 31.25 % (N = 15) of the mother-infant interactions were double-coded by independent raters that were blind to all other assessments. Intraclass correlations (ICCs) proved to be adequate before consensus scoring of disagreements on ratings (sensitivity, ICC = 0.87, cooperation, ICC = 0.98).

2.4.3. Maternal psychological distress

A psychological distress composite was created considering maternal psychopathological symptoms and the presence of maternal life stressors.

The Brief Symptom Inventory (BSI; Derogatis, 1983; Portuguese version by Canavarro, 1999) was used to evaluate maternal psychopathologic symptoms. The BSI comprises 53 items, rated on a 5-point Likert scale, covering nine symptom dimensions: Somatization, Obsession-Compulsion, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism; and three global indices of distress. In the current study, the Positive Symptom Distress Index (PSDI) was used, providing information about the average level of distress. This index is the sum of the values of the items receiving non-zero responses divided by the positive symptom total (PST), which represents a count of all the items with non-zero responses. The Portuguese validation study of BSI found it to be the best index to discriminate subjects with and without psychopathological problems (Canavarro, 2007). The Cronbach's alpha for internal consistency in the present study was α = 0.97.

The Daily Hassles Questionnaire (Kanner, Coyne, Schaefer, & Lazarus, 1981; Portuguese version by Negrão, Pereira, & Soares, 2010) was used to assess the stressors related to daily life and the activities of motherhood. Hassles are defined by irritating, frustrating everyday demands, that can be related to inner concerns (e.g., physical appearance), financial concerns (e.g., not enough money for basic necessities), and concerns related to the child/family (e.g., overloaded with family responsibilities; difficulty dealing with children's behavior in public places). This self-report measure includes 43 items, rated on a 5-point scale (0 = *no hassle* to 4 = *big hassle*). Items were summed to obtain a total final score. Internal consistency in the present study was α = 0.96.

The mean of the standardized scores of BSI and Daily Hassles Questionnaire were combined, resulting in a maternal psychological distress composite, since they proved to be significantly correlated ($p < .001$). Higher scores indicate increased maternal psychological distress.

2.5. Analysis plan

Data were analyzed with SPSS version 27.0. Preliminary analyses were performed to examine descriptive and bivariate correlations between the study variables and to investigate potential covariates of infant cortisol reactivity and negative affect. Two separate hierarchical regression models were then computed. The first model tested the predictors of infant cortisol reactivity, considering mother's sensitivity and cooperation, as well as child neonatal risk, family socioeconomic status, and maternal psychological distress. A second regression model was computed to examine the predictors of infant negative affect, considering all the previous variables, including infant cortisol reactivity.

3. Results

Descriptive statistics and correlations between study variables can be found in Tables 1 and 2, respectively. As shown in Table 2, maternal sensitivity and cooperation were both positively associated with infant's cortisol reactivity (ΔT1 − T2), showing that a decrease of infant's cortisol concentration in response to the dyadic interaction task was significantly associated with the exposure to more sensitive, $p = .024$, and less intrusive, $p = .047$, maternal behaviors. Note that a positive cortisol variation between T1 and T2 indicates a decrease in cortisol concentration. Infant negative affect was found to be negatively associated with maternal cooperation, suggesting that infants exposed to more intrusive maternal behaviors showed more expressions of infant negativity throughout the interaction with the mother. Infant negative affect was marginally associated with cortisol reactivity, $p = .053$. No significant associations were found between the control variables and the infant's cortisol reactivity and negative affect.

Tables 3 and 4 presents the results of the two hierarchical regression models. Given the consistent empirical findings showing that infant neonatal risk, family SES, as well as, maternal psychological distress, are linked to preterm children's development, all of these variables were included in the first steps of the two regression models as covariates. Maternal sensitivity and cooperation were included in the models in the final step 4 regarding PT infants' cortisol reactivity and negative affect. Cortisol reactivity was also included as a covariate in the second regression model aiming to predict infant negative affect.

Regarding infant cortisol reactivity, the overall model was not significant, $F(2,35) = 1.47$; $p = .22$; $R^2 = .13$, and no significant individual predictor emerged (see Table 3). Concerning infant negative affect, the overall model was not significant, $F(2,36) = 1.06$; $p = .40$; $R^2 = .20$ (see Table 4). In terms of individual predictors, maternal cooperation was shown to be the only significant variable associated with infant negative affect, $p = .03$, after controlling for infant neonatal risk and cortisol reactivity, as well as family SES and maternal psychological distress.

4Discussion

Preterm infants impaired ability to regulate their physiological stress responses have been increasingly described over the last years. Even though maternal quality of care is considered to be important for the regulation of the infant's physiological states (Gunnar & Quevedo, 2007; Hostinar et al., 2014), empirical investigation on this issue have still received little attention among preterm samples with most research previously conducted with full-term, healthy sample**.** The present study investigated changes in cortisol responses and infant negative affect during a structured mother-infant interaction task in infants born preterm.

4.1. Associations between PT infant's cortisol reactivity and maternal interactive behavior

Our results suggest a link between the quality of maternal care towards the infant during interaction and the infant's cortisol activity, which is in line with the general findings of previous research both with full-term and preterm samples (Albers et al., 2008; Bosquet Enlow et al., 2014; Brummelte et al., 2011; Tarullo et al., 2017). Univariate analysis indicated a decrease of infant's cortisol concentration throughout the interaction task if mothers were more sensitive and less intrusive in their behaviors. However, contrary

Table 2
Bivariate associations between study variables.

	Variable	1.	2.	3.	4.	5.	6.	M *(SD)*
1.	ΔT1 − T2 cortisol	–						-0.03 (0.33)
2.	Negativity	-0.28[a]	–					2.19 (0.96)
3.	Child Neonatal risk	-0.17	-0.08	–				-0.09 (0.89)
4.	Family SES	-0.17	0.18	0.09	–			1.13 (1.22)
5.	Maternal psychological distress	0.04	-0.02	0.26	0.45	–		-0.05 (1.88)
6.	Maternal sensitivity	0.33[b]	-0.17	0.11	-0.23	-0.07	–	4.58 (1.76)
7.	Maternal cooperation	0.29[b]	-0.32[b]	-0.03	-0.16	-0.13	0.621[c]	4.35 (1.62)

Note. Pearson and point-biserial correlations.
[a] p = .053.
[b] p < .05.
[c] p < .01.

Table 3
Summary of hierarchical linear analysis, predicting cortisol reactivity.

	R^2 (Adj R^2)	*β*	*t*	*p*
Step 1	0.01 (– 0.02)			
Child neonatal risk		-0.07	-0.47	0.64
Step 2	0.04 (– 0.01)			
Family SES		-0.18	-1.13	0.27
Step 3	0.05 (– 0.03)			
Maternal psychological distress		0.10	0.58	0.54
Step 4	0.13 (0.01)			
Maternal sensitivity		0.03	0.13	0.90
Maternal cooperation		0.29	1.33	0.22

Table 4
Summary of hierarchical linear analysis, predicting child negativity.

	R^2 (Adj R^2)	*β*	*t*	*p*
Step 1	0.07 (– 0.03)			
Child neonatal risk		-0.10	-0.65	0.52
Child cortisol reactivity		-0.26	-1.7	0.10
Step 2	0.10 (0.03)			
Family SES		0.16	0.99	0.33
Step 3	0.10 (0.00)			
Maternal psychological distress		-0.05	-0.34	0.74
Step 4	0.20 (0.06)			
Maternal sensitivity		0.32	1.4	0.18
Maternal cooperation		-0.46	-2.2	0.04

to expectation, once controlled for neonatal risk, family SES and maternal psychological distress, the associations were rendered non-significant.

It is important to note that some of the evidence produced in previous investigation did not take into account the influence of infant, maternal and environmental variables as controlled in our study, that are possibly also implicated in this process. Thus, a rigorous interpretation of our study and of previous research does not allow to affirm the existence of a direct causal relation between maternal behavior and infant cortisol regulation (Albers et al., 2008).

Other factors not considered in this study may be more strongly related with cortisol response in preterm samples. Specifically, previous studies indicated that maternal prenatal stress is related to altered infant cortisol responses (de Weerth, Buitelaar, & Beijers, 2013). It has been suggested that in utero-exposure to stress may shape the offspring's HPA-axis functioning with long-term consequences (Räikkönen, Seckl, Pesonen, Simons, & Van den Bergh, 2011). Thus, the exposure to maternal pre-natal stress may constitute a more pervasive factor of infant's physiological dysregulation later on development. It is all the more important among preterm studies considering research suggesting that preterm birth is associated with higher levels of perceived stress during pregnancy (Lima et al., 2018). In our study, current maternal perceived stress and psychopathological symptoms were not correlated with infant cortisol reactivity. It is important to note those indicators were assessed at 12 months postpartum and not prenatally, and this may account for differences in the results. Also, some research has suggested that is the stability of higher levels of maternal stress from the earliest months after birth to childhood that better differentiates children in the highest levels of cortisol response, from children never exposed to maternal stress (Essex et al., 2002).

In the present study, maternal behavior did not predict infant cortisol reactivity, which was calculated based on the difference between a baseline saliva sample and a second sample, collected after the interaction episodes ended. Although, we did not assess infant cortisol during an extended period of recovery. Previous studies suggested that this particular time-point, that enables to differentiate infants that retained higher cortisol levels for a longer period of time, may me more strongly related to maternal sensitivity and cooperation (Albers et al., 2008).

4.2. Associations between PT infant's negative affect and cortisol reactivity

This study also aimed to investigate the association between cortisol and behavioral reactivity, assessed in terms of infants' negative affect, during the same mother-infant interaction task. The association between infant negative affect and cortisol reactivity trended toward significance, such that higher levels of infant cortisol were marginally related to elevated expressions of negative affect during the interaction. However, regression analysis performed did not confirm the cortisol reactivity as a significant predictor of infants' negative affect. In this regard, although research on FT children has produced inconclusive findings, Jansen et al. (2010), in a review, state that most psychological stressors, including paradigms involving the mother as a possible source of stress, do not provoke a significant cortisol reaction, and so it is possible that physiological and behavioral systems are not synchronized. Further, the simultaneous activation of these two systems seems to decrease with age (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Lashansky et al., 1991). As children's behavioral repertoire becomes more sophisticated it is unlikely that many stressors provoke a significant cortisol response, as the behavioral actions turn to be more efficient in controlling the stressor (Jansen et al., 2010). This explanation is supported by systems compensation theory that states that, in healthy populations, correlations between physiological and behavioral responses should be less probable to occur since the activation of one system acts to compensate the other. For instance, children's manifestations of distress, such as crying, may lead to the early removal of the stress-causing stimulus, preventing significant changes in cortisol levels (Keenan, 2000). On the contrary, in at-risk samples, such as in the case of prematurity, systems may fail to regulate each other and the activation of both systems is more likely to happen (Habersaat et al., 2013). Therefore, as settled by Keenan (2000), dysregulation may occur when significant changes happen simultaneously in more than one system (e.g., physiological and behavioral). This is an interesting hypothesis to consider since the literature has already clearly established PT infant's difficulties in the regulation of both physiological and behavioral systems (Bilgin & Wolke, 2016; Clark et al., 2008; Feldman, 2009). However, our data was unclear as to whether this hypothesis can be confirmed. Only additional research, including a full-term control group, will afford to clarify this result. Nevertheless, it is noteworthy that our sample includes an important number of moderately-to-late preterm infants. Consequently, we can speculate that it somewhat approximates to the characteristics and behavior of healthy, low-risk samples, and that a stronger association between the two systems would have been found in a more uniform sample composed with very-to-extreme preterm infants. In line with this, Habersaat et al. (2013) found in preterm infants a positive correlation between only one element of emotion regulation and cortisol levels at a specific time point, not being enough to affirm the existence of a different pattern of relationship between these two systems, compared to the FT sample where no significant correlations were detected. In contrast, Erickson et al. (2013) did find an association between cortisol level and behavioral responses. Although, they found that elevations in cortisol responses were correlated to more expression of both negative and positive behavioral responses. Such result suggests that different affective reactions have a similar physiological expression, being unclear about the nature of the stimulus that provokes a simultaneous activation of behavioral and physiological systems, and the directionality of this relation, among PT infants.

It is important to note that, maternal intrusiveness was the only significant predictor of observed infant negativity. In line with this result, a wide range of studies with FT and PT infants investigating the impact of low quality of care has found an association between less positive maternal behaviors and more difficulties in terms of infant emotion regulation during the first years of life (Forcada-Guex et al., 2006; Heron-Delaney et al., 2016; Samdan et al., 2020). Particularly, maternal intrusiveness seems to increase infant negative interactive behavior and distress (Galligan et al. 2018; Perry, Dollar, Calkins, & Bell, 2018; Scaramella, Sohr-Preston, Mirabile, Robison, & Callahan, 2008). An intrusive mother may struggle to consider the baby as a separate and autonomous person, endowed with desires and intentional behavior, interfering frequently and abruptly cutting off the baby's activities. As a result, this pattern of interactive behavior may compromise the establishment of a synchronous relationship. Intrusive mother's, as they are not in tune with baby's needs and desires, may fail to serve as an adequate co-regulator of infant emotional experiences and behavior, resulting in dysregulated expression of affect (Egeland, Pianta, & O'brien, 1993; Perry et al., 2018).

On the other hand, considering the mutually interactive nature of the dyadic relationships that takes places between the mother and the infant (Bell, 1968), infant characteristics, such as negative temperamental dispositions, can also evoke less positive maternal behavior (Klein et al., 2018; Lengua, & Kovacs, 2005; Micalizzi, Wang, & Saudino, 2017). Maternal care does not perform isolated, and infant negative emotionality also acts as a relevant impelling force for changes in the dynamics of mother-infant interactions. More difficult infant's may lead to more difficulties for mothers to recognize their needs and address them in a proper manner. This is particularly relevant to consider given research that identified PT to be particularly negative and less regulated, which behavior is often disorganized, and challenging for parents to manage (Als, Butler, Kosta, & McAnulty, 2005; Cassiano, Gaspardo, & Linhares, 2019; Cassiano, Provenzi, Linhares, Gaspardo, & Montirosso, 2020; Hughes et al., 2002). Further, it is important to note that early infant negative affect disposition coupled with less adjusted parenting responses constitutes an exceptionally risk for later regulatory problems, as those factors are additive in their consequences (Crockenberg, Leerkes, & Bárrig Jó, 2008; Feldman, Greenbaum, & Yirmiya, 1999; Kim & Kochanska, 2012; Lengua, & Kovacs, 2005).

4.3. Limitations and further directions

There are limitations to this study that should be addressed in future research. The sample is relatively small, such that it was not possible to divide and compare infants into groups by gestational age and birth weight. Also, even though the inclusion of a wider range of gestational ages with infants being born moderately-to-late preterm is an advantage, as it allows us to obtain information from a group of PT infants frequently overlooked, at the same time introduces a larger heterogeneity in terms of neonatal and medical experiences that should be considered in data interpretation. Future research should examine the patterns of cortisol response and their relation to behavioral reactivity more specifically across different preterm gestational age groups, and introduce a FT control group. Such information is crucial, as enables the investigation of possible links between behavioral and physiological systems according to the degree of prematurity, and to design more accurate clinical interventions. Further, the observed correlations were generally weak and, due to the cross-sectional nature of the study, did not allow us to draw causal conclusions. Therefore, the results must be interpreted with caution and further longitudinal research is warranted.

However, despite such limitations, there are also several strengths. Firstly, this study contributed to advancing our understanding by considering simultaneously the influence of important neonatal and environmental variables on the cortisol responses of preterm infants. Besides, we examined both biological and behavioral PT infants' responses concurrently, during the same mother-infant interaction procedure. It is also noteworthy, that such procedure is closer to the daily interaction challenges that may spontaneously occur between the dyad, contrary to more artificial procedures often performed in research. Furthermore, we adopted a multimethod and multiple-informant perspective that yields a relevant richness to the study, as obtaining information from multiple sources and methods conveys more robust information of child functioning.

This study adds to the existing literature and have a potential to aware public health professionals to the difficulties that may arise in the mother-infant interaction patterns. Healthcare programs should incorporate more comprehensive assessments of infant's functioning and support parents in identifying and responding to the signals of their infants – premature in many ways – and whose behavior is often disorganized, and difficult to address. Early individualized intervention programs may be helpful to support parents to recognize the specific needs and competencies of their infant, as some strategies may be helpful to one infant and stressful to another. Furthermore, it is essential to be aware of the early signs of socio-emotional and behavioral difficulties, since can be associated with later psychopathology (Briggs-Gowan, & Carter, 2008; Delobel-Ayoub et al., 2009; Keenan, 2000).

CRediT authorship contribution statement

Vanessa Moutinho: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Funding acquisition. **Joana Baptista:** Conceptualization, Methodology, Resources, Supervision, Project administration. **Ana R. Mesquita:** Conceptualization, Methodology, Resources, Supervision. **Dieter Wolke:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision. **Carolina Toscano:** Methodology, Investigation, Data curation. **Carla Moreira:** Formal analysis. **Ana C. Bernardo:** Data curation. **Isabel Soares:** Methodology, Resources, Project administration.

Author Note

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Data Availability

Data will be made available on request.

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