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Attachment Security and HPA Axis Reactivity to Positive and Challenging Emotional Situations in Child–Mother Dyads in Naturalistic Settings

ABSTRACT: *This study investigated adrenocortical activity in response to different challenging and positive affect emotional contexts in child–mother dyads, as function of attachment security (children's secure base behaviors and mothers' attachment representations). Fifty-one children ranging in age from 18 to 26 months and their mothers participated in this study. Secure children showed significant increases in their cortisol levels after fear episodes and significant decreases, after positive affect ones. No significant changes were found for frustration/anger episodes. Insecure children did not show significant differences in cortisol levels in any of the episodes, which suggests that insecure attachment may be related to hypothalamic–pituitary–adrenal axis suppression in response to challenging and positive contexts. Mothers of insecure children showed significantly higher cortisol concentrations in pre- and post-session samples, than mothers of secure children. Mothers' personal attachment representations influenced their own cortisol responses, as well as their children's (in a marginal significant way). © 2011 Wiley Periodicals, Inc.* Dev Psychobiol

Keywords: *HPA axis; attachment; challenging and positive contexts; child–mother dyads*

INTRODUCTION

According to a biopsychological perspective (Calkins, 1994; Gross & Thompson, 2007; Loman & Gunnar, 2010; Thompson & Meyer, 2007), the comprehension of emotions requires the study of the physiological processes associated with emotional experience, as well as the understanding of the reciprocal and dynamic relationships between the biological and behavioral expressions of emotion. The hypothalamic–pituitary–adrenal (HPA) axis is one of the relevant physiological systems studied by researchers, nowadays. This occurs, mainly, due to two reasons (Stansbury & Gunnar, 1994): (1) adrenocortical activity is highly sensitive to emotional experience, particularly the regulation of stress responses. At the same time, emotions seem to mediate the intensity of the HPA axis response to stressful and challenging situations (Sapolsky, 1998, 2007); (2) easy and non-invasive measurement of cortisol (hormone released by the HPA system during stress) through small samples of saliva, both in adults and in children (Hanrahan, McCarthy, Kleiber, & Tsalikian, 2006; Kirschbaum & Hellhammer, 1994). In order to understand the reciprocal and psychobiological relationships between the HPA axis and emotions, most of the work is done using mild stressors and brief situations, (Fox, Cahill, & Zougkou, 2010; van Bakel & Riksen-Walraven, 2004) addressing, mainly, two

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questions: (1) which emotions activate the HPA stress response; (2) which emotion regulation strategies mediate this response (Lewis & Ramsey, 2002; Schieche & Spangler, 2005). The development of a secure attachment relationship between the mother and the child is considered one of the most important life-long strategies in reducing negative and sustaining positive emotions and their associated physiological processes, being considered as a buffer against stress (Bowlby, 1973; Bowlby, 1980; Stansbury & Gunnar, 1994). Animal and human research suggests that the release of cortisol by the HPA axis is sensitive to variations in quality of early care. Children with a secure attachment do not exhibit increases in cortisol levels when the attachment figure is present, unlike the insecure ones, more likely to show increases in the presence of the attachment figure (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998). However, the relationships between the parents' attachment representations and their children's adrenocortical reactivity to different emotional situations have not been studied before. This hypotheses seems relevant, given that mothers' personal attachment history and representations influence and predict their children's attachment security in a significant and trans-generational way (Main, Hesse, & Kaplan, 2005; van IJzendoorn, 1995) and there is a significant physiological attunement of maternal and child adrenocortical response to child challenge. Sensitive mothers seem to be physiologically more in tune with their children's cortisol responses, showing significant correlations between their adrenocortical activity and their children's, unlike the less sensitive ones, during the performance of a challenging task for children (Sethre-Hofstad, Stansbury, & Rice, 2002).

When it comes to which emotions activate the HPA stress response, there is a scarcity of research work on individual differences in cortisol reactivity, both in adults and in children. In this study, we examined the relationship between attachment security and HPA axis reactivity in response to challenging (fear and frustration/anger) and positive affect situations, both in children and in mothers. Fear responses involving novel and uncertain events are associated with cortisol increases (Gunnar, Marvinney, Isensee, & Fisch, 1989), namely, during maternal separation episodes in 9 (Gunnar, Larson, Hertsgaard, Harris, & Broderson, 1992a) and 18-month-old infants (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). Some studies have also examined the relationship between frustration/anger and HPA axis stress responses in children. There seems to be an overactive HPA axis stress response associated with reactive aggression (defense and hypersensitivity to perceived threats), but no significant relationships between proactive aggression (instrumental, goal oriented, and planned) and cortisol (van Bokhoven et al., 2005). Reactive aggressive children showed higher cortisol reactivity than proactive and non-aggressive children, after being exposed to fear and frustration eliciting tasks (Lopez-Duran, Hajal, Olson, Felt, & Vasquez, 2009). Finally, the exposure to novel events that elicit positive affect (mother–infant swim classes) seems to decrease cortisol in infants, especially in the mothers' presence (Hertsgaard, Gunnar, Larson, Brodersen, & Lehman, 1992; Gunnar & Donzella, 2002). Research on the regulation of positive affect is essential, particularly, the study of strategies that maintain and increase the experiences of positive emotions, since cultivating them may be particularly important for building resilience to stressful events (Tugade and Fredrickson, 2007).

OBJECTIVES

The objective of this work is to study adrenocortical reactivity during episodes of fear, positive affect, and frustration/anger in the context of mother–child dyads and its relationships with attachment (children's secure base and mothers' attachment representations).

We hypothesized that: (1) Children and mothers' HPA axis stress responses to different emotion-eliciting contexts will vary as function of children's attachment relationship quality. Secure children and their mothers are expected to show no significant differences after the exposure to negative affect/stressful episodes and significant decreases after positive affect situations. Insecure dyads are expected to show significant cortisol increases after challenging contexts and significant decreases after positive affect situations.

(2) Children and mothers' HPA axis stress responses are expected to vary as function of mothers' personal attachment representations, given that mothers' personal attachment history and representations influence and predict their children's attachment security in a significant and trans-generational way (Main et al., 2005; van IJzendoorn, 1995) and there seems to be a significant physiological attunement between children and mothers, as function of maternal sensitivity (Sethre-Hofstad et al., 2002). Secure mothers and their children are expected to exhibit no significant differences after the exposure to negative affect/stressful episodes. Insecure mothers and their offspring are expected to show cortisol increases after challenging episodes. During positive affect episodes, both secure and insecure mothers, as well as their offspring, are expected to show significant decreases in cortisol levels.

METHODS

Participants

Fifty-one child–mother dyads (26 boys and 25 girls), all Caucasian, from bi-parental families participated in the study. Children's age ranged from 18 to 26 months of age (M = 21.33; SD = 1.96). They spent from seven to 11 hr (M = 6.89; SD = 2.71) at day-care each weekday. Mothers' age ranged from 25 to 43 years (M = 33.64; SD = 4.10) and fathers' age from 26 to 55 years old (M = 35.88; SD = 5.86). Mothers' level of education ranged from 9 to 19 years (M = 15.18; SD = 3.04) and fathers' from 4 to 19 years (M = 13.92; SD = 3.62). Participants represented a range of socioeconomic status backgrounds, as reflected by parental education and were recruited from public and private daycare centers. All participants were healthy at the time of assessment and there were no premature children.

Materials and Procedures

All procedures were carried out with the adequate understanding and written consent of the participants (mothers).

Emotion Regulation Paradigm: Fear, Positive Affect, Frustration/Anger. An emotion regulation paradigm (Diener & Mangelsdorf, 1999), composed by three episodes, designed to elicit emotionally challenging (fear and frustration/anger) and positive affect reactions in children was presented. The episodes were elicited by presenting children with three different toys. Each episode lasted for 6 min. If children showed 30 s of sustained high-intensity distress, mothers were instructed to become involved.

All stimuli used in this work were previously tested in a pilot test in a group of 10 children, showing a varying emotional intensity in most children. During the frustration/anger episode, we presented children with a movable box with wheels, shaped as a yellow bear, which contained colored lego pieces inside. After the experimenter judged that the child was involved with the toy (2 min on average), the experimenter took the toy away firmly and placed it out of reach but within the child's sight. During fear episodes, a dinosaur toy with elements of novelty, unpredictability and intrusiveness was used to elicit fear. Finally, during the positive affect episode, children were given a toy piano that played music and created musical rhythms. Similar procedures and toys were used in other studies (Buss & Goldsmith, 1998; Diener & Mangelsdorf, 1999; Grolnick, Bridges, & Connell, 1995). The predominant emotion showed by children during the three episodes was coded, in order to test if the emotional manipulations were effective and if the target emotion was expressed more frequently in the correspondent episode, than the other emotions in a significant way. *Fear* was scored when the child expressed at least one of these facial features: eyebrows raised or drawn together; eyes wide; mouth open, corners straight back. *Positive affect* was scored when the child smiled or produced a positive vocalization (laugh). *Anger* was coded when the child showed at least one of the following: brows pulled back down or together; raised cheeks; straight or angular mouth; or tight lips. A score of ''neutral'' was given when the child did not express any of these emotions and showed a neutral expression. Neutral scores were not included in the analysis. The child's quality of emotion was coded during 15-s interval. If the child expressed more than one emotion during the time intervals used for coding, the most intense emotion was coded as the predominant one in a scale of 1 (mild intensity) to 3 (extremely intense) for each 15-s interval. Extremely intense emotion could be expressed by facial affect, body postures, gestures and movements, or full intensity vocalizations (e.g., laughter for positive affect; crying or screaming for negative affect). Low-intensity effect seemed mild and would be more ambiguous than high intensity one. To obtain emotional expression scores we added the number of 15-s intervals each child expressed fear, frustration/anger, and positive affect as the predominant emotion (Diener & Mangelsdorf, 1999).

Separate pairs of coders, blinded to the hypotheses, coded the three episodes. Inter-rater reliability was calculated using Cohen's Kappas (fear = .73; positive affect = .84; frustration/anger = .70). This coding system is similar to those used in other studies of children coping strategies (Buss & Goldsmith, 1998; Calkins and Johnson, 1998; Diener & Mangelsdorf, 1999; Nachmias et al., 1996).

The emotion regulation episodes were videotaped at the family's house in different days, in order to avoid any emotional contamination from one episode to the other. They all started at the same time (18:30 h). The time chosen to start the experiments was late afternoon, because 96% of the mothers worked outside the home and finish their shift around 17:00 h. The three episodes were videotaped in a counterbalanced way in order to control any order effect over the results.

Attachment Behavior Q-Set (AQS, Version 3.0). The Attachment Behaviour Q-set (AQS), (Waters, 1995) evaluates the quality of the child's secure base behavior towards the mother or other figures in an ecologically valid context, namely, the children's home, during a period of 2 h. The 90 items of this instrument are distributed on a scale of 9 points, ranging from ''extremely characteristic'' to ''extremely uncharacteristic.'' Mothers became aware of this work through an informed consent, left at their children's daycare. The AQS home visits were scheduled with the mother in a time of day when any other members of the family or friends were present at home. The visits were conducted by two observers that were trained not to disturb interactions in progress or interfere in domestic routines. The observers' agreement was analyzed through Spearman Brown correlations, which mean was .80. Individual Q-sorts, resulted from a mean between the descriptions of the two observers. Children final attachment score was obtained through a Pearson correlation between the child's individual Q-sort and the security criterion value of the ''ideal child'' (Waters, 1995; Waters & Deane, 1985). This correlation represents the place occupied by children on a security continuum. This value ranges between −1.0 and 1.0. Children who are able to use the mother or other figure as a secure base receive a higher value, while the least able to do it, receive lower values. In most normative samples, security scores average about .35 (Bost, 2006). The coders of infant attachment behaviors were in blind to maternal attachment status. This study uses the AQS for child attachment, instead of the Strange Situation (Ainsworth, Blehar, Waters, & Wall, 1978) procedure. Both measures are used in the field and both have proved to be valid measures to access quality of attachment. The validity of the AQS using observers, but not self-reported, has been clearly confirmed in a meta-analysis (van IJzendoorn, Vereijken, Bakermans-Kranenburg, & Riksen-Walraven, 2004) and it was included in the same category, in terms of quality, as that of the Strange Situation and the Adult Attachment Interview. Previous studies with Portuguese samples supported the utility and validity of the AQS in the Portuguese culture (Veríssimo, Monteiro, & Santos, 2006; Veríssimo, Monteiro, Vaughn, Santos, & Waters, 2005). Also, and very important, the Strange Situation is not recommended for the age level of our participants (Ainsworth et al., 1978).

Adult Attachment Representation Narratives. The "Adult attachment representation narratives" (Waters & Rodrigues-Doolabh, 2004) is an instrument developed to gain access and analyze adult attachment representations and secure base scripts in possible daily and anxious scenarios related with the attachment relationship. The secure base script is described by a series of events which defines the attachment relationship in terms of a balance between proximity towards the attachment figure and exploration behaviors, shown by the child or the adult. These events are: (1) the secure base (parent or partner) supports one's exploration; (2) the secure base remains available and responsive in case of need; (3) a threatening conflict and obstacle appears, which leaves the individual feeling anxious and fearful; (4) the individual searches and looks for comfort in the secure base and/or the secure base comforts the individual; (5) the conflict and threat are resolved; (6) proximity and contact with the secure base comforts the individual in an effective way and helps him/her to deal with the resulting anxiety; (7) the individual returns to his/her initial activity or changes it in a tranquil way (Posada et al., 1995). There are six narratives (four stories with attachment content and two used for control purposes). In each one of them are presented four groups of suggestive words, developed to guide the production of the narratives. The narratives are scored on a 7-point scale. The highest values are assigned when the script is elaborated, reveals knowledge and sensitivity concerning the emotional state of others and reinterprets the meaning of the obstacle/conflict (suggested by the group of words) in a positive way. The narratives were assessed in a counterbalanced way, in a separate day from the AQS home visits and emotional situations and were scored by two trained observers, blinded to the results of the Q-sort home observations. Rater agreement was calculated as the intraclass correlations across rater-pairs. The intraclass correlations ranged from .64 to .85 with over 85% of scores being within 1-point scale. Spearman–Brown reliability estimates story groups ranged from .83 to .95. The final score for each story was the average across raters. An overall score was calculated by averaging scores over all the stories. Cronbach's alpha's for the overall scores were .84.

In this study, we used the Narratives instead of the Adult Attachment Interview (AAI), (George, Kaplan, & Main, 1984). Both the AAI and the narratives are valid measures in the study of attachment. Even if the secure base script is a recent measure, the validity has been clearly demonstrated. Correlation between AAI results and the Narratives' results are high. Several articles already showed that the measure is stable and correlated (as the AAI) with the quality of attachment between mother and child (Coppola, Vaughn, Cassibba, & Costantini, 2006; Vaughn et al., 2006, 2007).

Children and Mothers' Salivary Cortisol Levels. The cortisol response was assessed from saliva, using Sarstedt's salivette kits in mothers and Salimetrics' sorbettes in children. All saliva samples were frozen at $-80°C$ within 2 h after the collection. The samples were centrifuged (3,000 rpm) at $10°C$, during 20 min. The assessment of cortisol was done by using luminoimmunoassay (LIA) kits (IBL, Hamburg, Germany). The mean intra- and inter-assay coefficients of variation were 5.5% and 6.8%, respectively. During each episode, children and mothers' salivary cortisol samples were collected two times, immediately before the episode (pre-session sample 1) and 30 min after the end of the session (post-session sample 2). To control for effects of assessment time on cortisol due to cortisol circadian rhythm, control samples were also collected in a different day at 18:30 h (sample 1) and 30 min after (sample 2), in both members of the dyad. The collection of control samples was counterbalanced in order to control anxiety or habituation variables around the saliva collection procedure. Half of the control samples were collect 2 days before the emotion episodes started. The other half was collect 2 days after the episodes ended. The number of control samples is only 13, due to logistic reasons related to difficulties in collecting extra samples. All the procedures were developed at the children's homes, when the mothers where taking a break from their daily tasks. Many of them refused to collect extra samples, given the extra inconvenience. Therefore, 13 samples were collected, which proven to be sufficient to show no significant differences. The samples were analyzed at the Integrative Behavioural Biology Lab at ISPA-Instituto Universitário.

Statistical Analysis

A repeated measures MANOVA was conducted to examine any possible significant differences in children's salivary cortisol responses, as function of children's attachment security. Children's cortisol levels were the dependent variables and children's attachment security was the independent variable. This analysis included two within-subjects factors: emotion episode (fear, frustration/anger, positive affect) and sampling moment (pre-session; post-session). For use as between-subjects factor, children's attachment security was dichotomized. The participants were grouped according to their scores on the AQS, into participants with secure (score $\geq .35$) versus insecure (score $< .35$) attachment (Bost, 2006). The AQS methodology allows the distribution of the data in a continuum or dichotomic way (Bost, 2006). The authors considered that dichotomizing the attachment security data would be more useful and clear in understanding the cortisol variation, especially, since there are two different and fixed cortisol analysis moments (pre- and post-session).

In order to examine significant differences in mothers' salivary cortisol responses, as function of children's attachment security, a repeated measures MANOVA was undertaken. Mothers' cortisol levels were the dependent variables and children's attachment security was the independent variable. This analysis included two within-subjects factors: emotion episode (fear, frustration/anger, positive affect) and sampling moment (pre-session; post-session). For use as between-subjects factor, children's attachment security was dichotomized in a similar way as described above (Bost, 2006).

In order to examine any possible significant differences in children's salivary cortisol responses, as function of mothers' attachment representations, a repeated measures MANOVA was conducted. Children's cortisol levels were the dependent variables and mothers' attachment representations were the independent variable. This analysis included two within-subjects factors: emotion episode (fear, frustration/anger, positive affect) and sampling moment (pre-session; post-session). For use as between-subjects factor, mothers' attachment representations were dichotomized. Mothers were grouped according to their total results on the "Adult attachment representation narratives," into participants with secure (score $\geq$3.5) versus insecure (score $<$3.5) attachment representations (Waters & Rodrigues-Doolabh, 2004; Waters & Waters, 2006).

Finally, in order to examine significant differences in mothers' salivary cortisol responses, as function of mothers' attachment representations, a repeated measures MANOVA was undertaken. Mothers' cortisol levels were the dependent variables and mothers' attachment representations were the independent variable. This analysis included two within-subjects factors: emotion episode (fear, frustration/anger, positive affect) and sampling moment (pre-session, post-session). For use as between-subjects factor, mothers' attachment representations were dichotomized in a similar way as in the children's MANOVA (Bost, 2006).

When the MANOVAs results were significant, relevant differences were tested with planned contrast estimates analyses.

RESULTS

Preliminary Analyses

First, we examined cortisol values for outliers, that is, values $>$3 *SD* above mean. In children, three participants (no. 19, 25, 48), during frustration/anger episodes showed outlier values and were taken out of the analysis. In mothers, two of them (no. 28, 30) had missing values in attachment representations total scores and one mother (no. 41) had two missing post-session cortisol values. Therefore, for these analyses, the final sample sizes for the attachment security groups were 31 secure and 17 insecure children; 30 secure and 16 insecure mothers.

Next, we tested if the emotional manipulations were effective and if the target emotion was expressed more frequently in the correspondent episode, than the other emotions in a significant way. A repeated measures MANOVA was conducted. Two within-subjects levels were used: emotional expression (fear, positive affect, and frustration/anger facial expressions) and episode (fear, positive affect, frustration/anger). Child gender served as between-subjects or independent variable. The analysis revealed significant main effects for episode [F (2, 106) = 37.94, $p < .001$] and emotional expression [F (2, 106) = 7.35, $p < .001$]. A significant interaction between episode and emotional expression was also found [F (4, 212) = 80.36, $p < .001$]. No child gender effects were found.

Planned contrast estimates analyses revealed that during fear episodes (Tab. 1), children showed more fearful expressions, than positive affect [t (46) = 2.44, $p < .05$] and more fear than frustration/anger expressions [t (46) = 9.73, $p < .001$]. During positive affect episodes, they showed more positive affect expressions, than fearful ones [t (46) = 6.13, $p < .001$] and more positive affect than frustration/anger expressions [t (46) = 7.30 $p < .001$]. Finally, during the frustration/anger episodes, children expressed more frustration/anger faces, than positive affect ones [t (46) = 7.20, $p < .001$] and more frustration/anger expressions than fearful one [t (46) = 17.11, $p < .001$]. Thus, the manipulations were effective and the stimuli elicited the emotion they were designed to evoke.

Next, control samples were analyzed and paired t-tests were conducted. No significant differences between control sample 1 and 2 (30 min later) were found for either children [t (13) = .01, $p > .05$] or mothers [t (13) = .34, $p > .05$].

No significant sex differences were found for cortisol measures or children's attachment (AQS scores). Correlational analyses revealed no effects for parents' age or level of education and the number of hours spent by children at daycare each day, on children and mothers' adrenocortical measures.

Finally, a significant bivariate correlation was found between children's attachment security (AQS scores) and mothers' attachment representations ($r = .64$, $p < .01$).

Effects of Attachment Security (AQS) on Children's Cortisol Responses, in Episodes of Fear, Positive Affect, and Frustration/Anger

In children's case, we found no significant main effects for attachment security. However, a significant interaction episode $\times$ sampling moment $\times$ children's attachment security was found [F (2, 92) = 3.08, $p = .05$].

Table 1. Means and Standard Errors for Children's Emotional Expressions (Fear, Positive Affect, Frustration/Anger), During Episodes of Fear, Positive Affect, and Frustration/Anger

Episode	Emotional Expression	*M*	*SE*
Fear	Positive affect	3.78	.62
	Frustration/anger	.28	.10
	Fear	6.60	.62
Positive affect	Positive affect	5.04	.54
	Frustration/anger	.73	.17
	Fear	.75	.26
Frustration/anger	Positive affect	2.27	.29
	Frustration/anger	6.14	.36
	Fear	.00	.00

In secure dyads, no significant differences between secure children's pre-session samples were found, except between fear and positive affect episodes, [t (46) = 2.02, $p < .05$], (Fig. 1a). Secure children showed significant increases in cortisol levels during fear episodes [t (46) = 2.90, $p < .01$, Fig. 1a] and significant decreases during positive affect episodes [t (46) = 2.67, $p = .01$, Fig. 1a]. No significant differences were found in frustration/anger episodes. Secure children's cortisol reactivity changed significantly across episodes. Significant differences were found between secure children's cortisol reactivity to fear and positive affect episodes, [t (46) = 4.17, $p < .001$] and between fear and frustration/anger episodes [t (46) = 2.05, $p = .05$]. Secure children's cortisol increased in response to fear episodes and was non-significant in response to frustration/anger ones (Fig. 1a).

In insecure dyads, insecure children (Fig. 1b) did not show any significant differences between pre-session samples or significant differences between pre- and post-session samples in any of the three episodes.

When it comes to differences between secure and insecure children, no significant differences between the two groups were found in pre- or post-session samples for any of the three episodes. However, significant differences were found between secure and insecure children's cortisol reactivity to fear episodes [t (46) = 2.22, $p < .05$]. Secure children's cortisol variation increased in response to fear episodes (Fig. 1a) and was non-significant in insecurely attached ones (Fig. 1b). No significant differences were found for positive affect or frustration/anger episodes.

Effects of Attachment Security (AQS) on Mothers' Cortisol Responses, in Episodes of Fear, Positive Affect, and Frustration/Anger

In mothers, a significant main effect for sampling moment was found [F (1, 44) = 23.36, $p < .001$].

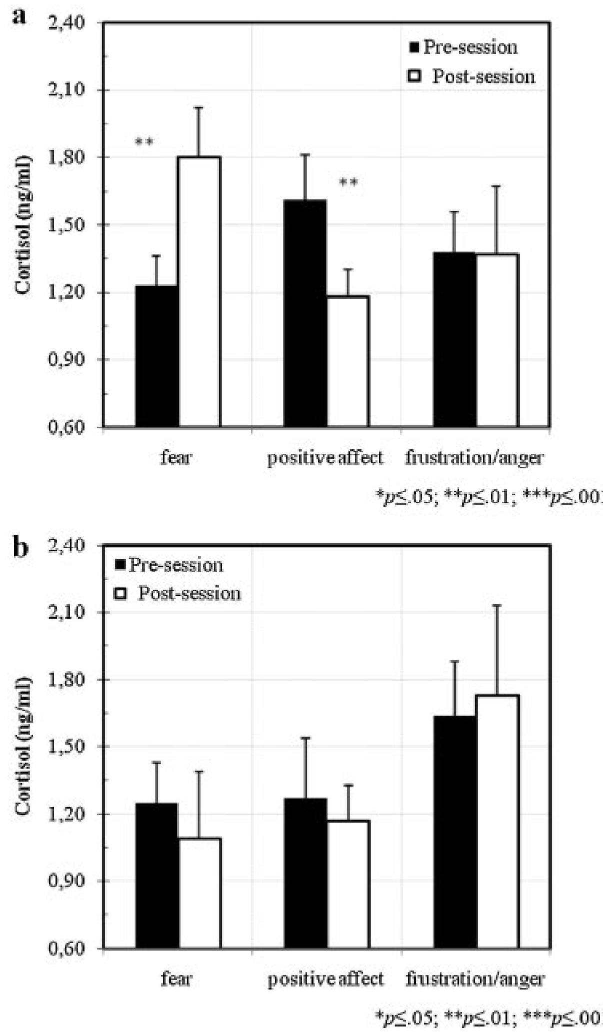

FIGURE 1 Means and standard errors for secure (a) and insecure (b) children's cortisol levels (ng/ml), as function of emotional episode and sampling moment.

Most importantly, a significant main effect for children's attachment security [F (1, 44) = 6.06, $p < .05$] and a significant interaction sampling moment × children's attachment security [F (1, 44) = 4.81, $p < .05$] were also found in mothers.

No significant differences between pre- and post-session samples were found for mothers of secure and insecure children. Insecure children's mothers (Fig. 2) showed significantly higher cortisol concentrations in pre-session samples, than mothers of secure children [t (44) = 2.55, $p = .01$]. In post-session samples (Fig. 2), insecure children's mothers showed significantly higher cortisol concentrations in post-session samples, than mothers of secure children [t (44) = 2.21, $p < .05$].

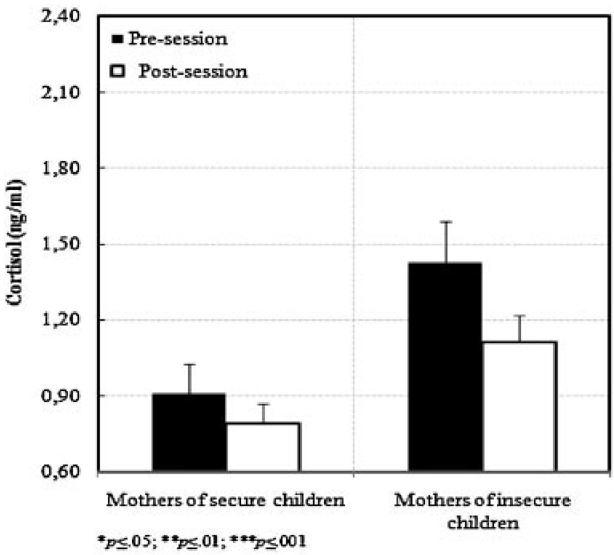

FIGURE 2 Means and standard errors for mothers' cortisol levels (ng/ml), as function of children's attachment security (AQS) and sampling moment.

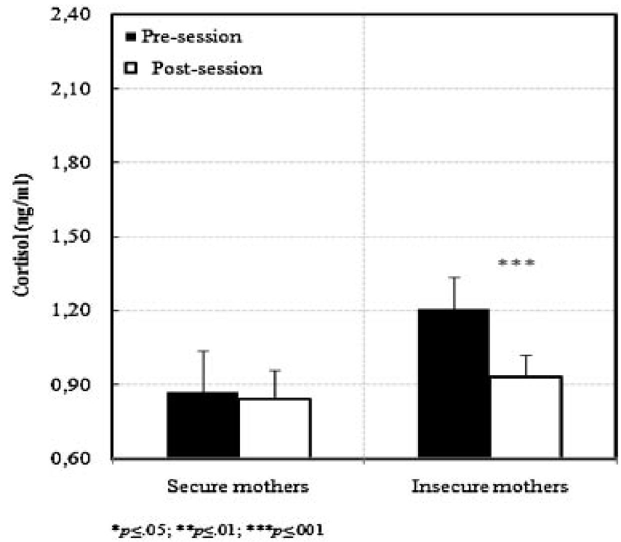

FIGURE 3 Means and standard errors for mothers' cortisol levels (ng/ml), as function of mothers' attachment representations and sampling moment.

Effects of Mothers' Attachment Representations (Narratives) on Children's Cortisol Responses, in Episodes of Fear, Positive Affect, and Frustration/Anger

In the case of children's cortisol responses, a marginal significant interaction episode $\times$ sampling moment $\times$ mothers' attachment representations [F (2, 88) $= 2.86$, $p = .06$] was detected.

Effects of Mothers' Attachment Representations (Narratives) on Mothers' Cortisol Responses, in Episodes of Fear, Positive Affect, and Frustration/Anger

When it comes to mothers' cortisol responses, a significant main effect for sampling moment was found [F (1, 42) $= 11.47$, $p = .001$] and most importantly, a significant interaction sampling moment $\times$ mothers' attachment representations [F (1, 42) $= 7.66$, $p < .01$] was also found.

Secure mothers (Fig. 3) showed no significant differences between pre- and post-session samples. Insecure mothers (Fig. 3) showed a decrease in their cortisol levels from pre- to post-session [t (42) $= 5.10$, $p < .001$]. When it comes to differences between secure and insecure mothers, no significant differences were found between the two groups in cortisol pre- or post-session samples.

DISCUSSION

Similar to other studies (Dettling et al., 2000; Loman & Gunnar, 2010), children's adrenocortical responses were significantly influenced by the quality of care. In this study, secure children showed significant increases in their cortisol levels after fear episodes and significant decreases, after positive affect ones. No significant changes were found for frustration/anger episodes. These findings are similar to other studies, which reported that responses involving novel and uncertain events result in cortisol increases (Gunnar et al., 1992a; Nachmias et al., 1996) and pleasurable activities decrease cortisol levels, especially in the mothers' presence (Gunnar & Donzella, 2002; Hertsgaard et al., 1992). Children's non-significant cortisol responses to frustration/anger episodes, could be associated with the proactive aggression tendencies (to get the toy back), shown by children during this episode (Lopez-Duran et al., 2009). On the other hand, insecure children's HPA axis did not show any significant differences in its reactivity, after the exposure to positive or negative episodes. These findings contrast with previous studies (Gunnar et al., 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998), which showed that securely attached children do not exhibit increases in their cortisol levels, unlike insecure ones, more likely to show increases. These authors reported that attachment security works as a buffer against stress. In contrast, our results suggest that insecure attachment may be associated with HPA axis suppression during challenging and positive emotional contexts in insecure children, associated with past and continuous rejection experiences with the attachment figure, which may have cause habituation of the adrenocortical stress response. In fact, even though negative emotions have been associated with the activation of the HPA response, research has shown that a rapid adaptation and habituation of the cortisol response, after repeated exposure to a psychological stressor is highly characteristic in humans, namely children (Gunnar, Hertsgaard, Larson, & Riagatuso, 1992b; Gunnar et al., 1989). In fact, some insecure children (called avoidant in the Strange Situation procedure) tend to show a suppressive or minimizing emotional and behavioral expression style, when compared to securely attached children, not showing overt distress during negative contexts or pleasure on reunions (Lutkenhaus, Grossmann, & Grossmann, 1985; Malatesta, Culver, Tesman, & Shepard, 1989; Spangler & Grossman, 1993), which seems to have an adaptive and regulatory effect by reducing rejection experiences (Bowlby, 1980). On the other hand, securely attached children tend to show an ''open and flexible emotion expression style,'' characterized by a coherent demonstration of expressions of joy during pleasurable situations and the experience of negative emotions, during stressful events (Cassidy, 1994), which may explain the significant cortisol increases during fear episodes and decreases, during positive affect ones. This open communication style occurs in secure children because, unlike insecure children, a sensitive and ameliorative response is expected by the attachment figure (Cassidy, 2008). In contrast to the Strange Situation procedure, the AQS does not present a differentiation between insecure avoidant and insecure ambivalent children, characterized by opposite styles of emotion expression, which is a limitation in this study. Avoidant children tend to show a minimizing expression style in order to avoid rejection. On the other hand, ambivalent children display heightened negative emotionality and exaggerated fearfulness, as a way to gain the attention of an insufficiently or inconsistently available parent if true danger appears (Main, 2000; Main, Kaplan, & Cassidy, 1985). These results highlight the importance of assessing attachment security through different but complementary methodologies, such as the AQS and the Strange Situation. In the future it would be interesting to replicate this study using the Strange Situation and compare it with the results of this work.

Mothers of insecure children showed significantly higher cortisol concentrations in pre-session samples, than mothers of secure children. It seems that insecure children's mothers were more distressed with the possibility of exposing themselves and their children to different emotional contexts than mothers of secure children. On the other hand, mothers' of secure children probably had a higher perception of control over the events, built on past secure attachment experiences, characterized by children's cooperation and mothers' effectiveness (Bowlby, 1980; Cassidy, 1999), which might explain the lower pre-session cortisol levels.

In this study, results also showed that mothers' attachment representations not only predicted their children's attachment quality, but also influenced both their own cortisol responses, as well as their children's (in a marginal significant way) cortisol responses. In fact, the results showed that secure mothers' cortisol responses did not show any significant changes after from pre-session to post-session samples, probably due to a strong perception of control and effectiveness over the events, based on their personal past secure attachment experiences. On the other hand, insecure mothers exhibited a significant decrease in their cortisol concentrations from pre- to post-session sampling moments. It seems that insecurely attached mothers might have been distressed about the events prior to the sessions, due to personal past attachment experiences, which might have diminished their perception of effectiveness and security in controlling events. In fact, insecure mothers show more difficulties and anxiety in understanding correctly their children's communication signs, either by distorting or blocking them, which causes them to behave in a rejecting and/or unpredictable way towards their children (Hesse, 1999; Main et al., 2005; van IJzendoorn, 1995).

This work was developed at the children's homes and not at the laboratory. Differences between naturalistic versus controlled settings may play an important role in the differences found between the results of this study and the ones reported in others (Gunnar et al., 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998). The results of this study suggest that past results on insecure children's increased adrenocortical stress responses, may be associated with being on a strange controlled environment (laboratory), rather than with attachment quality alone. The attachment system is described in terms of a balance between proximity towards the caregiver and exploration behaviors, shown by the child or the adult. This balance is described by a sequence of events organized in an emotional and mental script, called the secure base script, developed in early infancy and internalized by the individual across development, including adulthood (Posada et al., 1995). In this sense, we can assume that the attachment relationship can be observed at home. Moreover, The AQS was designed specifically to examine individual differences in secure base-behavior, covering a broad range of secure base and exploratory behavior, affective responses, social referencing and other aspects of social cognition, all observed at home, presenting an overview of the entire domain of attachment relevant behavior, during stressful situations and exploration/playful ones (Bost, 2006). In sum, these differences in results suggest, once again, the importance of using different but complementary methodologies in the study of attachment, in order to clarify these issues.

In future studies it would be very interesting to study possible gene-mediating effects, since these ones may help us to understand how emotional experiences may change the individual's responses to future stressful events, given that many of these effects influence memory and the integration of new information.

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