

# **Paper III**

# Maternal soothing and infant stress responses: Soothing, crying and adrenocortical activity during inoculation

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## Abstract

The relation between maternal soothing and infant stress response during inoculation was examined in a sample of 37 mothers and their 3-month-old infants. The mothers' soothing and the infants' cry vocalizations and the mothers' and the infants' salivary cortisol level pre- and post-injection were analysed. There was a positive relation between infants' cry vocalization post-injection and maternal soothing pre- and post-injection. The sample was divided in two sub-groups depending on whether the mothers evidenced most soothing of the infants in the period before (Preparatory group;  $n=20$ ) or after (Contingent group;  $n=17$ ) the syringe injection. In the Preparatory group, the duration of infant cry vocalizations was related to amount of maternal soothing before and after the injection, while cry vocalizations in the Contingent group was related to amount of maternal soothing after the injection. The Contingent infants responded to the injection with a significant increase in cortisol, while there was no increase in the Preparatory infants. The Preparatory infants evidenced significantly longer duration of looking at the target stimuli in a visual marking task, suggesting greater difficulties in disengaging attention. These findings indicate that 3-month-olds' stress responses and their mothers' situational behaviour are mutually regulated.

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## 1. Introduction

Infants' responses to painful stimuli are found to vary considerably (e.g. De Weerth, van Geert, & Hoijsink, 1999; St. James-Roberts, Conroy, & Wilsher, 1998), reflecting individual differences both in distress crying (Barr, 1998) and activity of the hypothalamic–pituitary–adrenocortical (HPA) axis (Gunnar & Donazella, 1999). The social context and in particular the caregivers' situational behaviour is also acknowledged to play an important role in this variability (e.g. Ainsworth, Blehar, Waters, & Wall, 1978). Contingency and promptness of the caregivers' responses to infant crying is thus considered to play a crucial role in regulating infants' level of distress (e.g. Ainsworth et al., 1978; Bowlby, 1988).

In a naturalistic study of 26 infant–mother pairs, Bell and Ainsworth (1972) assessed the long-term effects of maternal consistency and promptness in responding to infant crying and concluded that differences in the infants' crying by the end of the first year could be attributed to the history of contingent responses to infant crying rather than individual differences in irritability among the infants. However, Gewirtz and Boyd (1977a) later claimed that Bell

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and Ainsworth's (1972) measures of maternal responsiveness and infant crying were inadequate and confounded (see Ainsworth & Bell, 1977; Gewirtz & Boyd, 1977b for a further discussion). Even though Gekoski, Rovee-Collier, and Carulli-Rabinowitz (1983) found that the infants' duration of crying decreased over the first 6 months as an effect of maternal soothing, this was not a unique effect to maternal soothing, and it also had an adverse effect on renewed crying. In an effort to extend the Bell and Ainsworth (1972) study Van IJzendoorn and Hubbard (2000; see also Hubbard & Van IJzendoorn, 1991) found in fact that the more frequently mothers ignored their infants' crying in the first 9-week period, the less frequently these infants cried at the subsequent 9-week interval.

Other studies have examined the effect of maternal soothing on alleviating infant distress to specific aversive events, such as inoculation. While Lewis and Ramsay (1999) found no relation between maternal soothing and infant cortisol and behavioural responses to inoculation, both Sweet and McGrath (1998) and Axia and Weisner (2002) found that amount of maternal soothing was associated with longer duration of crying in 5–6-month-olds. However, in these studies the mothers' situational behaviour were only assessed after the injection, which makes it difficult to disentangle what is cause and effect (see Emde, 1992).

In addition to investigating the possible alleviating effects of maternal soothing on infant distress, the inoculation situation is also interesting from a social referencing (e.g. Feinman, 1982) or a mood modification (e.g. Campos, 1983) perspective, where infants' emotional responses are suggested to reflect the infants' referential understanding of how their caregivers relate to objects and situations (Moses, Baldwin, Rosicky, & Tidball, 2001). A classic example is that infants' willingness and reluctance to cross a visual cliff is found to be attributable to which facial emotions their caregivers were instructed to pose before the infant is exposed to the cliff (Sorce, Emde, Campos, & Klinnert, 1985). In accordance with this, a number of studies in older children have demonstrated that their responses to painful medical examinations are related to their caregivers' situational behaviour just prior to the event (Bush, Melamed, Sheras, & Grennbaum, 1988; Frank, Blount, Smith, Manimala, & Martin, 1995).

Social referencing is assumed to develop at the end of the infant's first year of life (Feinman & Lewis, 1983), but this contrasts findings from recent studies of face-to-face interaction, which have shown that 2–3-month-olds are sensitive to social contingency in interaction with their mothers (see Murray & Trevarthen, 1985; Nadel, Carchon, Kervella, Marcelli, & Réserbat-Plantey, 1999; Stormark & Braarud, 2004). A possible explanation for why studies suggest that social referencing skills develop later is that it has typically been examined in terms of infants' locomotion, which precludes the demonstration of social referencing in younger infants. In addition, the influence of caregivers' emotional reactions has primarily been studied in artificial, rather than natural emotional situations (Emde, 1992), where the infants have to rely on the adults' facial expression rather than physical contact which constitutes much of early emotional communication between infants and caregivers (Ainsworth, 1992).

The purpose of this study was to examine the relation between 3-month-olds' stress responses to inoculation and their caregivers' situational behaviour before and after the syringe injection. We observed the duration of infant distress crying after the syringe injection and maternal situational behaviour both before and after the stressful event. The mothers' responses to the inoculation were indexed by amount of pre- and post-injection soothing. In Norway, the standard procedure of infant inoculation is that it is the caregiver, and not the nurse, who holds the infant before, during and after the injection. This allowed us to observe the interaction between infants and caregivers during inoculation in a naturalistic observation design (see Negayama, 1999), and not just examining the alleviating effects of soothing.

Salivary cortisol samples were drawn from the infants and mothers who were the ones who accompanied them during the inoculation before and after the injection. Heighten concentration of cortisol reflects increased activation of the HPA-system (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995), but note that the HPA system is a non-specific response system, since it responds to both endogenous and exogenous stressors, including psychosocial stressors (Gunnar & Prudhomme White, 2001; Schore, 1997).

It was expected that the infants would evidence a significant increase in level of cortisol after compared to before the injection (see Gunnar, Hertsgaard, Larson, & Rigatuso, 1992; Lewis & Thomas, 1990). For the mothers it was assumed that the anticipation of the inoculation could lead to an equally elevated level of cortisol as when the mothers witnessed the inoculation of the infant (see Smyth et al., 1998). Finally, infants' duration of distress crying after syringe injection during inoculation has been found to be associated with the efficacy to inhibit attention (see Axia, Bonichini, & Benini, 1999). We included data from a visual marking experiment where the infants were tested for their attentional control of exogenous stimuli (Harman, Posner, Rothbart, & Thomas-Thrapp, 1994) to assess individual differences of reactivity and self-regulation which is considered to be a temperament factor (Rothbart & Derryberry, 1981).

## 2. Method

### 2.1. Subjects

A total of 41 mother–infant dyads participated in the study. Four infants started crying before the injection and the data on these dyads were excluded from statistical analyses. The final sample thus comprised 37 dyads with mothers (mean age: 29 years; range: 19–39 years) and infants (mean age: 13 weeks and 4 days; range: 12–17 weeks); 19 boys and 18 girls with no history of antenatal, natal or postnatal problems. All infants were in good health conditions. Thirty-five mothers were married or co-habitants and two mothers were single mothers. The educational level of the mothers was obtained as an index of socioeconomic status: Nine had earned master degrees, 10 held bachelor degrees, 17 had graduation from high school and 1 mother had graduation from 9-year compulsory school as their highest level of completed education.

### 2.2. Apparatus and procedure

The mothers were recruited to the study by the nurses at the health care centres, who informed the mothers about the longitudinal project on emotional development which this study is part of. The mothers and the infants met with the first author (HCB) in relation to the visual marking experiment when the infants were around 10 weeks old.

During the visual marking experiment the infants sat in a baby car seat in front of three adjacent computer monitors placed next to each other, with a horizontal distance of 30° from the infant. They were presented with a total of 24 trials. Each trial started with a 2500 ms presentation of a fixation stimulus (flashing stimulus) on the centre monitor, followed by a 2500 ms bilateral (left or right monitor) presentation of either a face or geometrical figures which served as the attention cue (see *Axia et al., 1999*), followed by a 1000 ms presentation of the fixation stimulus on the centre monitor, and finally, the presentation of the target which was a bilateral presentation of faces or geometrical figures on left and right monitor for 5000 ms.

A video camera, placed on top of the centre monitor, captured the infant's eye movements and a mirror reflection of what was presented on the monitor. This made it possible to determine the extent to which the infants' gaze was contingent upon presentation of the stimuli on the monitors. In this study we estimated the infants' attentional control in terms of percentage amount of time looking at the target stimuli during the 5000 ms target period.

At the health care centre the participants first met with HCB to draw samples of pre-injection saliva. Then they met with the nurse, who administered the inoculation against diphtheria, pertussis and tetanus (DPT) and *Haemophilus influenzae* type b (HiB). The serum was injected in the infants' thigh while sitting on the mother's lap (in one case on the accompanying father's lap). Time of the day for the injection varied from 08:00 a.m. to 01:30 p.m. None of the infants were fed or received pain analgesics in relation to the inoculation. The sequence from the start of the consultation until the infant had calmed down after the injection was video-recorded using a digital video camera (JVC GR-DVL 107). Then HCB left the nurse's office. When 27 min had passed since the injection, the post-injection saliva samples were drawn from the mother and the infant to assess the peak response to the inoculation (Gunnar, personal communication).

The saliva samples in both the pre- and post-injection periods were collected with "Salivettes" (Sarstedt, Inc., Nümbrecht, Germany). The Salivette included a neutral cotton swab, a small suspender inserted in a plastic centrifuge vessel and a stopper on the top to close the Salivette. Saliva was collected by placing the absorbent dental cotton roll which was applied to the tongue, cheeks and gums of the subjects. The saturated cotton roll was placed into the Salivette and put into a refrigerator, before storing it in a deep freezer at the laboratory.

During the period of data collection, the protocol changed from a twin- to a single-injection immunization. Thus, eight of the infants received twin-injections, while the rest of the final sample (29) received a single injection. For one of eight twin-injection infants, the interval between the twin injections was sufficient to allow the infant to calm down, thus the infants' responses to the first injection could be calculated in isolation. The remaining seven received the second injection before they had calmed down after the first injection, and for these infants duration of crying was calculated in relation to the second injection. Calculations of possible differences between the single- and the twin-injection infants showed that there were no differences in length of crying between the single- and the twin-injection group. There was no differences in level of cortisol, neither pre-injection nor post-injection, which is in accordance with *Ramsay and Lewis' (1994)* comparison of stress responses in infants receiving single or multiple injections.

### 2.3. Data analyses

#### 2.3.1. Behaviour data

All video recordings of the behavioural data were scored using the The Observer Video Pro version 3.0, where both the infants' cry vocalization and the mothers' soothing behaviour were scored continuously on a frame-by-frame basis, with a temporal resolution of 41.67 ms. Inter-rater agreement was calculated separately for each behaviour category between two coders and ranged from .91 to .99 for maternal soothing and infant cry vocalizations and .83 for infant visual gaze in the visual marking task.

Data of six infants in the sample was missing due to technical problems, and these subjects were thus excluded from the statistical analyses of the infants' attentional control.

#### 2.3.2. Infant cry vocalizations

Infants' duration of crying was scored from onset of cry vocalizations after the syringe injection to offset with no renewed crying within the next 10 s. Facial expressions were not scored since many of the infants and their mothers engaged in front-to-front contact, which made it difficult to see the infants' facial expressions on the video-recordings. This definition of behavioural quieting resembles that of studies on calming effects of sucrose (e.g. Blass & Watt, 1999) and differs from the affect scores used by Lewis and colleagues (e.g. Lewis & Ramsay, 1999), but the two measures are found to correlate (Worobey & Lewis, 1989).

#### 2.3.3. Maternal soothing

The mothers' physical contact and gaze at infant during the inoculation procedure was scored. These behavioural indices are found to be important in regulating affective states in infants (see Schore, 1994; Trevarthen, 1979). Since all of the mothers held their infants during the inoculation, physical contact was scored only when the mothers' actively used holding, rocking, touching, kissing, hugging, frontal–frontal contact to increase the bodily proximity with their infants. On the basis of the maternal physical contact and eye-to-eye contact with the infant an overall soothing index was calculated, separately during the pre- and post-injection intervals, and then made to an average soothing score, ranging from 0 to 1, where a maximum score would mean that the mothers soothed their infants all the time during the interval. Two coders scored 36 of the inoculation video files and five of the visual marking task files.

#### 2.3.4. Cortisol data

The analyses of the salivary cortisol samples were carried out according to the radioimmunoassay (RiA) principle with the Coat-a-Count commercial kit (Diagnostic Products Corporation, CA, USA) and assayed in the same batch. The intra-assay coefficient of variation was between 1.8% and 6.3% with detection limit at .15 nmol/l. A log 10 transform of the data was conducted to reduce skewness of the data (see Gunnar & Prudhomme White, 2001). Three dyads were excluded from the analyses of cortisol due to insufficient amount of saliva.

### 2.4. Statistical analyses

Pearson's  $r$  was conducted to assess the relation between mothers' soothing behaviour pre- and post-injection and infants' stress responses and attentional control.

## 3. Results

### 3.1. Relation between soothing and stress responses

There was a significant positive correlation between infant cry vocalization and maternal soothing pre-injection ( $r = .34, p < .05$ ) and post-injection ( $r = .61, p < .001$ ) (see Fig. 1). There was also a significant positive correlation between maternal soothing pre- and post-injection ( $r = .47, p < .01$ ; see Fig. 1).

There was no significant correlation between the infants' attentional control and stress responses (cortisol pre-injection or cortisol and crying post-injection). However, infants' attentional control were correlated with maternal soothing pre-injection ( $r = .38, p < .05$ ), but not post-injection.

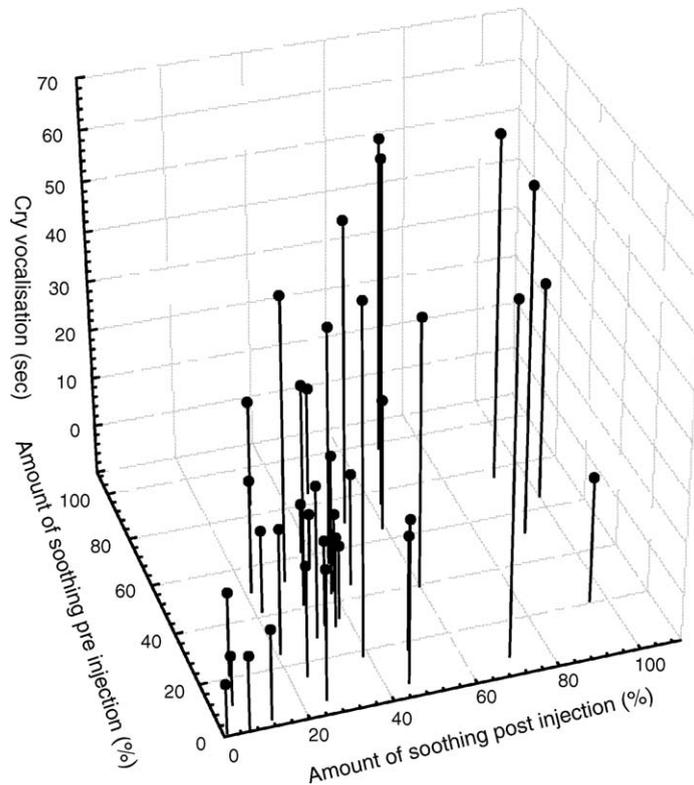


Fig. 1. A three-dimensional scatterplot of the correlation between maternal percentage amount of soothing pre- and post-injection and the infants' duration of crying post-injection.

As can be seen in Fig. 1 there was considerable individual variation both in level of soothing pre- and post-injection. Interestingly, most of the mothers, 20 of 37, showed a decrease in soothing in the pre- to the post-injection period. These mothers and their infants were referred to as Preparatory group since the mothers evidenced more pre- than post-injection soothing. The other 17 of the mothers evidenced more soothing after than before the injection and were thus referred to as Contingent mothers and infants.

There was a significant positive correlation between infants' post-injection crying and mothers' pre-injection soothing ( $r = .50, p < .05$ ) and post-injection soothing ( $r = .61, p < .01$ ) in the Preparatory group. In the Contingent group there was a significant relation between the infants' crying and the mothers' post-injection soothing ( $r = .61, p = .01$ ). There was a significant positive correlation between maternal soothing pre- and post-injection for both the Preparatory ( $r = .84, p < .001$ ) and the Contingent ( $r = .70, p < .01$ ) mothers, which reflects that the mothers had an individual stability in amount of soothing within the sub-groups irrespective of if their responding adhered to a contingent or a preparatory pattern of soothing.

The data on cortisol and maternal soothing were then subjected to separate two-way split-plot ANOVAs involving Group (Preparatory versus Contingent) and Period (Pre-injection versus Post-injection). To assess possible differences between the two groups of infants in amount of crying after the syringe injection and their efficacy to inhibit attention during the visual marking task, a one-way ANOVA was conducted involving Group (Preparatory versus Contingent).

### 3.2. Stress responses in the two sub-groups of infants and mothers

There was no significant difference in duration of crying between the Preparatory ( $\mu = 20.26$  s) and the Contingent infants ( $\mu = 26.7$  s).

For the infants' level of cortisol there was a significant main effect of period ( $F(1, 34) = 9.42, p < .01$ ), reflecting an increase in cortisol level post-relative to pre-injection. However, the Contingent infants had a significant increase

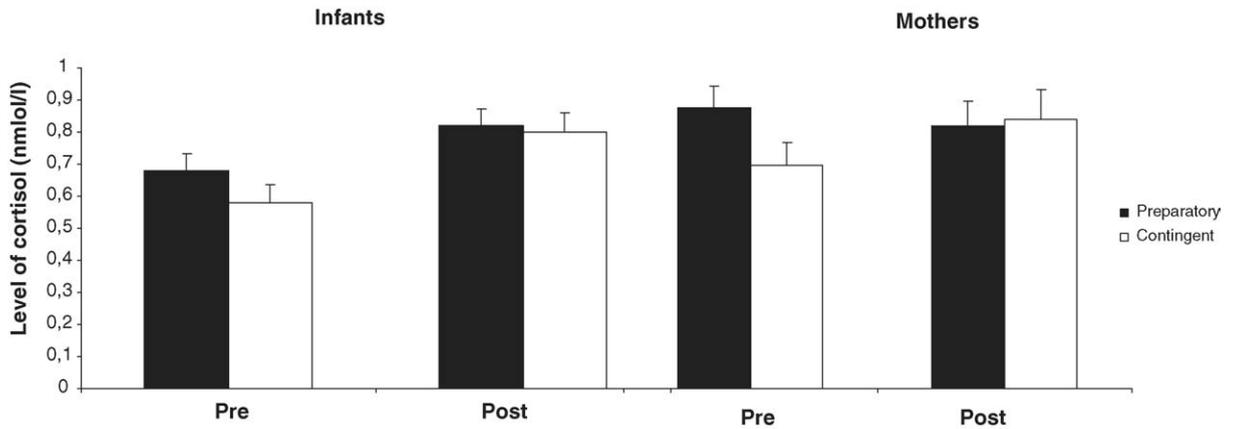


Fig. 2. Mean proportional level of cortisol in the two sub-groups of groups of infants and mothers: pre- and post-inoculation.

from pre- to post-injection levels of cortisol ( $F(1, 34) = 6.36, p < .01$ ; see Fig. 2), while there was no such difference in Preparatory infants. There were no significant main effects of Group or Period for the mothers' cortisol level.

### 3.3. Soothing in the two sub-groups of mothers

There was no overall differences in amount of soothing between the two sub-groups of mothers ( $F(1, 35) = 1.65, p = .21$ ). However, there was a significant two-way interaction of Group  $\times$  Period ( $F(1, 35) = 46.77, p < .0001$ ; see Fig. 3) reflecting that the Contingent mothers evidenced a significant increase ( $F(1, 35) = 30.51, p < .001$ ) and the Preparatory mothers a significant decrease ( $F(1, 35) = 16.80, p < .01$ ) in maternal soothing after compared to before the injection. The Preparatory mothers expressed significantly more soothing than the Contingent mothers in the pre-injection ( $F(1, 35) = 10.18, p < .01$ ), but not the post-injection period.

### 3.4. Attentional control in the two sub-groups of infants

The Preparatory infants looked significantly longer on the target stimuli in the visual marking task than the Contingent infants ( $F(1, 28) = 6.40, p < .01$ ).

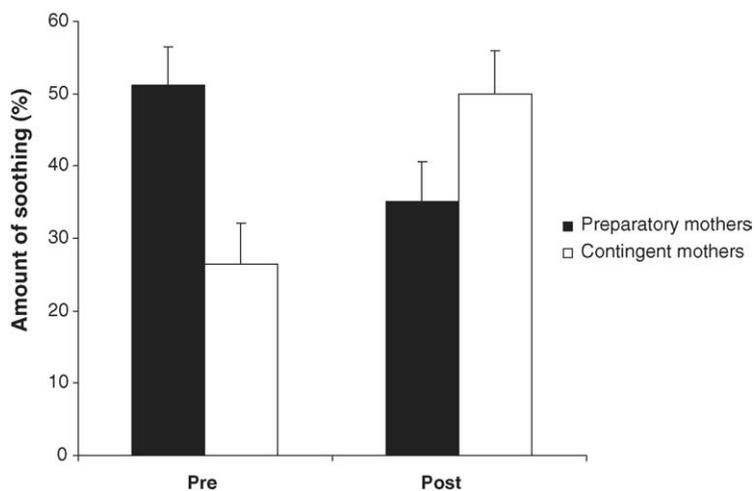


Fig. 3. Mean proportional maternal soothing in sub-groups of groups of mothers: Contingent mothers and Preparatory mothers: pre- and post-injection.

#### 4. Discussion

The main finding in this study was that infant cry vocalization was positively related to maternal soothing, not only after the injection but also before. The first finding accords with Sweet and McGrath (1998) and Axia and Weisner's (2002) finding that amount of maternal soothing was associated with more, not less crying in the infants. However, this finding could simply reflect that the mothers adjusted their behaviour to the infants' stress responses (see also Emde, 1992) rather than that the infants' crying was a function of the mothers' soothing per se.

Of more interest is the finding that amount of infant crying also was related to the mothers' amount of soothing before the injection, which suggests that infant stress responses to the inoculation is – at least to some extent – a function of the behaviour of their caregivers' anticipatory behaviour before the stressful event. This accords with Bush et al. (1988) and Frank et al. (1995) who found that young children's coping during medical examination was related to their caregivers' situational behaviour before the examination, studies demonstrating social referencing or mood modification in infants (Campos, 1983; Moses et al., 2001) and also in experiment of observational learning of fear response in rhesus monkeys offspring (e.g. Cook & Mineka, 1989). In all these studies it is suggested that infants' or young children's responses to novel stimuli are modulated by how their parents or other adults appreciate an actual emotionally aversive event.

In contrast to Axia et al. (1999), the infants' efficacy to inhibit attention in the visual marking task was not found to be related to their stress responses during the inoculation, instead it was related to the amount of maternal pre-injection soothing. One could speculate if this is attributable to the difference in amount of mother–infant contact during the syringe injection. The infants' in Axia et al.'s (1999) study received the syringe injection on an examination table and was probably to a lesser extent in contact with their caregiver during the inoculation than the infants in the present study. It is possible that this difference in procedure would put less demand on the infants' self-regulating skills, and greater demand on the caregivers' behaviour, which probably is influenced by the caregivers' past experience with the infants' regulatory capabilities.

A further inspection of the data revealed a variation among the mothers in soothing pattern, with what appeared to be two sub-groups of mothers and infants in the relation between maternal soothing and infant stress responses. The differences between the Contingent and Preparatory group did not involve main effects of neither the infants nor the mothers' responses to the injection, but how they responded before the injection and the relation between the pre- and post-injection responses. This was reflected in that the Preparatory mothers demonstrated significantly more pre-injection soothing than the Contingent mothers, and that the Preparatory infants evidenced no increase in cortisol from pre- to post-injection as the Contingent infants did.

In the Contingent group, infant cry vocalization to the injection was related to amount of maternal post-injection soothing. In the Preparatory group, infant cry vocalization was related to maternal pre-injection and post-injection soothing.

The correlation between maternal soothing and infant cry vocalizations in both groups suggests that infant stress responses and maternal soothing should be understood in relational terms rather than as simple stimulus–response patterns. This was further substantiated by the fact that amount of pre-injection maternal soothing was found to be highest among mothers whose infants evidenced elevated levels of pre-injection cortisol and indicated to have more problems inhibiting attention to the target in the visual marking task (e.g. McCall, 1994).

The findings from this study suggest that the distinction between the Contingent and the Preparatory group reflect how both biological and context-dependent maternal and infant responses to distressing events are mutually regulated rather than the extent to which maternal soothing alleviates infant distress. It is well established that care-giving responses in mothers are influenced by the infants' emotional responses (e.g. Emde, 1992), the mothers' awareness of the infants' temperament (Gunnar et al., 1995; Mangelsdorf, Gunnar, Kestenbaum, Lang, & Andreas, 1990) and even the mothers' general experiences with how the infants tend to respond to emotional stimuli (e.g. Legerstee & Varghese, 2001). So, even if it is natural to consider an alleviating effect of sensitive and contingent caregiver behaviour in response to the infants' expressions of distress, maternal soothing is probably also influenced by expectations of the forthcoming event, their history with their infant from associated experiences, and the infants' emotional state both prior to and during the injection. The differences in the infants' attentional control suggest that the Preparatory infants would evidence stronger reactivity than the Contingent infants, which could mean that the Preparatory infants had more problems with self-regulation and that their mothers therefore performed more soothing prior to injection.

The association between the participants' behaviour and cortisol was low. It could be argued that if both cortisol and crying are indices of a unitary stress response there should be a positive correlation between these sets of variables. However, studies (see Lewis, Ramsay, & Kawakami, 1993 for a review) have demonstrated that this is often not the case, in accordance with what has been referred to as the "problem of dissociation" (e.g. Gunnar & Donzella, 1999).

For the Preparatory group, one could speculate whether the lack of an increase in cortisol after the injection in the Preparatory infants reflects that the elevated level of pre-injection soothing in the Preparatory mothers actually had a stress-reducing effect in the infants. However, none of these findings were statistically significant and the elevated pre-injection cortisol level could also be argued to constrain the Preparatory infants' cortisol responses to the injection (see Gunnar & Prudhomme White, 2001).

The fact that neither the Contingent nor the Preparatory mothers evidenced any significant increase in level of cortisol after compared to before the injection may be understood in accordance with suggestions that anticipation of aversive events like for the mothers to witness distress in their infants may elicit an equally elevated level of cortisol as the event itself (Smyth et al., 1998).

Ramsay and Lewis (2003) have demonstrated that there are considerable individual differences among infants in their latency for peak cortisol responses. It is not unlikely that such individual differences could be important in assessing infant cortisol responses to a specific event like the syringe injection. In subsequent studies, the sampling of the participants' post-injection saliva should extend to multiple registrations to control for individual variations. A limitation of the present study was also that it did not control for the possible contribution of individual differences in infants' sleep-wake cycle feeding or other events that occurred prior to the arrival at the health care centre, which could have affected the pre-injection cortisol level of infants and parents (see Gunnar, Broderson, Krueger, & Rigatuso, 1996). It should also be emphasized that measures of cortisol during infancy reflects both infant characteristics and characteristics of the caregiving environment (Gunnar & Prudhomme White, 2001), which accords with the results in this study.

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