Emotion Regulation of 6-Months Old Infants during the Still-Face Paradigm: (The) Effect(s) on the Autonomic Nervous System

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Abstract

The aim of the present study was to examine infant emotion regulation, expressed in autonomic nervous system-reactivity, during the Still-Face Paradigm (SFP). In addition, the effect of maternal risk status on children's emotional and behavioural development was examined. The sample consisted of 51 mothers and their 6-month-old infants. Measures of heart rate, pre-ejection period (PEP), skin conductance level (SCL), and respiratory sinus arrhythmia (RSA) were collected during baseline and during the SFP episodes. Infant behavioural responses were coded as well. The SFP was able to elicit sympathetic and parasympathetic activity. In response to the still-face an increase in sympathetic activity was found, but only by SCL and heart rate, not by PEP. In addition, the still-face elicited an inhibition of the parasympathetic nervous system, the RSA decreased and the heart rate increased. In the transition from still-face to reunion, an effect of risk status was found on the pattern of heart rate and SCL. Infants from the high-risk group showed more sympathetic activity, indicating more stress and less emotion regulation. Overall, the SFP is able to elicit physiological features of emotion regulation and is able to indicate early differences in the autonomic nervous system activity in response to stress. Future studies should replicate these findings and should further investigate the role of maternal risk status.

Keywords: emotion regulation, still-face paradigm, autonomic nervous system, pre-ejection period, respiratory sinus arrhythmia, skin conductance, heart rate

Emotion Regulation of 6-Months Old Infants during the Still-Face Paradigm: (The) Effect(s) on the Autonomic Nervous System.

Antisocial behaviour has a large impact on society and is associated with extensive costs (Millie, 2009). The theory by Moffitt (1993) describes two trajectories for antisocial behaviour: a life course-persistent trajectory of antisocial behaviour and a trajectory with a peak in antisocial behaviour during adolescence. Moffitt theorised that the interaction between neuropsychological vulnerabilities and adverse environments is associated with the life-course persistent pathway of antisocial behaviour. Early-developing individual differences interact with the environment, which makes it difficult to change antisocial behaviour later in life. Therefore studies have focused on preventing antisocial behaviour by searching for developmental precursors to childhood psychopathology (Beelmann & Raabe, 2009; Piquero, Farrington, Welsh, Tremblay, & Jennings, 2009). Emotion regulation, which includes the ability to modulate, inhibit, and enhance emotional experiences and expressions (Calkins & Hill, 2007), appears to be associated with later behavioural problems and could therefore be a suitable candidate in the search of developmental precursors (Calkins & Dedmon, 2000; Gilliom, Shaw, Beck, Schonberg, & Lukon, 2002; Keenan, 2000; Supplee, Skuban, Shaw, & Prout, 2009). The current study will focus on emotion regulation during infancy. The emotion regulation will be specifically investigated by examining the reactions of the autonomic nervous system during a social interaction task. The influence of environment will be taken into account by comparing the results for infants with different risk status. Overall, the current study will contribute to the literature of preventing antisocial behaviour.

First emotion regulation will be discussed. Logically, emotions play an important role in emotion regulation. Emotions arise when an individual attends to a situation and sees it as relevant to his or her goals (Gross & Thompson, 2007). The meaning that the individual gives to the situation, leads to a certain emotion and when this meaning changes, the emotion is also likely to change. Additionally, emotions are whole-body phenomena: they are impulses which make individuals (re)act in certain ways and these impulses are associated with autonomic and neuroendocrine changes and subsequent behavioural responses. Moreover, emotions are malleable. Emotions can interrupt what we are doing and force themselves on our awareness, which is when conscious emotion regulation comes into play (Gross & Thompson, 2007).

Still-Face Paradigm

Emotion regulation by infants can be assessed using the still-face paradigm (SFP) designed by Tronick and colleagues (Tronick, Als, Adamson, Wise, & Brazelton, 1978). The procedure uses a three-step face-to-face interaction with the infant and an adult, often the mother. In the procedure the expected social norms of mother-infant interactions are violated, and infant emotion regulatory strategies and dyadic interactive characteristics can be assessed (Conradt & Ablow, 2010). Infant responses are assessed during the (1) play episode, in which the mother interacts with her infant as she would usually do, (2) the still-face episode, in which the mother remains immobile and freezes her face, and (3) the reunion episode in which the mother re-engages with her infant. Previous studies have shown that the SFP is able to elicit behavioural responses and physiological responses from infants (Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009)

Behavioural Reactions to the SFP

The SFP elicits the so-called still-face effect: infants show increased gaze aversion, less smiling and more negative affect during the still-face episode compared to the play episode (Gusella, Muir, & Tronick, 1988; Kisilevsky et al., 1998; Toda & Fogel, 1993). The overall pattern of the still-face paradigm has been investigated extensively and has also been confirmed in a meta-analysis (Mesman et al., 2009). The meta-analysis showed the classic pattern with reduced positive affect and gaze, and increased negative affect during the still-face paradigm. Additionally, a partial carry-over effect of the still-face effect was found during the reunion. Infant positive affect goes up, but does not reach levels as high as during the play-episode. Similarly, infant negative affect decreases but does not return to baseline levels. The meta-analysis also showed that the effects occur, regardless of gender, risk status and procedural variations.

Next to affect and gaze, studies have also focused on self-soothing behaviour and arching or squirming. These are coping strategies that are used to deal with stress. Self-soothing behaviour and attentional distraction are strategies to down-regulate (decrease) negative emotions, while arching/squirming is a strategy to up-regulate (increase) negative emotions, by kicking with the legs and banging with the arms (Ekas, Lickenbrock, & Braungart-Rieker, 2012).

Autonomic Nervous System Reactions to the SFP

The SFP is able to elicit reactions of the autonomic nervous system in infants (Conradt & Ablow, 2010). This is the case, because the autonomic nervous system (ANS) contributes to emotional responses (Kreibig, 2010). The ANS consists of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The sympathetic nervous system is responsible for physiological arousal and prepares the body for fight of flight, whereas the parasympathetic nervous system is responsible for resting and digesting (Costanzo, 2010). Interactions between the SNS and the PNS may have an effect on children's externalizing behavior (El-Sheikh et al., 2009). Specifically, co-activation or co-inhibition of the PNS and SNS was a vulnerability factor, whereas coordinated action served as a protective factor. The effects of activation ANS during stress can be measured using noninvasive methods. Heart rate, pre-ejection period, skin conductance and respiratory sinus arrhythmia are usable indices of the ANS and will be discussed below.

Heart rate. The cardiovascular system is relatively well-developed at birth and easy to record (Brownley, Hurwitz, & Scheiderman, 2000). Heart rate reflects the influence of the different branches of the nervous system. The SNS is responsible for acceleration of heart rate, while the PNS is responsible for deceleration. Previous studies have found that baseline levels of heart rate were associated with antisocial behaviour: Antisocial children had a lower baseline heart rate in comparison with control children and a lower baseline heart rate was associated with more antisocial behaviour in the future (Ortiz & Raine, 2004). Besides baseline heart rate, heart rate reactivity and regulation have been predictive of later cognitive and behavioural functioning. Heart rate variability is related to individual differences in attention, cognition, and emotion in children and adults (Fox, Schmidt & Henderson, 2000). Therefore infant cardiovascular responses to stress are an important topic of interest, because this regulatory behaviour establishes the response to stress in the future (Conradt & Ablow, 2010).

During the SFP, the heart rate usually accelerates during the still-face episode (Conradt & Ablow, 2010). The course of heart rate after the still-face episode is not entirely clear. Some studies report that the infants become less aroused and that their heart rate returns to baseline (Bazhenova, Plonskaia, & Porges 2001; Weinberg & Tronick, 1996), while other studies report more cardiac arousal during the reunion (Moore & Calkins, 2004). Others found that the effects were different, depending on the maternal sensitivity of mother and the infants resistance (Conradt & Ablow, 2010). Overall, studies have indicated that individual differences in heart rate levels during the SFP are indicators of regulatory capacities of infants and dyadic interactive history between mother and infant (Bazhenova et al., 2001; Haley & Stansbury, 2003; Moore & Calkins, 2004; Weinberg & Tronick, 1996).

Pre-ejection period. The pre-ejection period (PEP) is solely influenced by the sympathetic nervous system (Cacioppo et al., 1994; De Geus & Van Doornen, 1996; Oosterman, 2010). The pre-ejection period is the interval between the onset of left ventricular depolarization and the beginning of the ejection of blood in the aorta (Fox, Schmidt, & Henderson, 2000). In response to stress, the interval shortens and therefore the PEP decreases.

There are no studies to date that have measured the PEP of infants during the SFP. Nonetheless, there have been studies that used PEP as an indicator for emotion regulation. In a study that used the Strange Situation Procedure, in which mother and child (3- to 6-year olds) were separated and reunited, there was no effect of the procedure on PEP (Oosterman & Schuengel, 2007). However, in another study with the Strange Situation Procedure, there was a decrease in PEP during the separation, but only for young children with disorganized attachment relationships (Oosterman, 2010). Furthermore, there have been studies that used PEP reactivity to distinguish between groups. In middle childhood, it is possible to distinguish children with high externalizing behaviour from controls and from children with high internalizing behaviour through measuring PEP reactivity (Boyce et al, 2001): children with high externalizing behaviour show less reactivity to stress. In addition, PEP reactivity can also be used to distinguish groups with a comorbidity of ADHD and conduct disorder, from groups with only ADHD or from controls (Beauchaine, Katkin, Strassberg,& Snarr, 2001). The comorbid group displayed less PEP reactivity in comparison with the other two groups. In sum, PEP may play an important role in emotion regulation and may thus also be interesting to examine during the SFP.

Skin conductance. A second measure of the sympathetic nervous system is skin conductance, often reported as skin conductance level (SCL). Increased SCL reactivity is predictive of externalizing, internalizing and cognitive problems for elementary school girls. For boys of similar age internalizing problems could be explained by increased SCL reactivity (El-Sheikh, 2005). Low SCL reactivity is an important predictor in the (severity) of antisocial behaviour and in the onset of aggression in children. Low SCL reactivity in one-year olds during a stressor was able to explain aggression two years later (Baker, Shelton, Baibazarova, Hay, & van Goozen, 2013). The effect of SFP on infant SCL has not yet been investigated thoroughly. In publications, only Ham and Tronick (2006) investigated this effect and found an overall increase of SCL during SFP.

Respiratory sinus arrhythmia. According to the polyvagal theory (Porges, 2007), vagal regulation is associated with social affiliation behaviours, such as social interaction, behaviour, emotion, and attention (Moore, 2010). During periods of rest, vagal tone is activated. Vagal tone slows the heart rate down and allows a focus on internal, homeostatic processes (Moore, 2009) and is indexed by the Respiratory Sinus Arrhytmia (RSA) (Moore, 2010).

Baseline levels of RSA are related to stress: the higher the baseline level RSA the better the capacity of the individual to react adequately to a stressor (Porges, 1992). Beauchaine (2001) proposes that high levels of resting RSA during infancy is related to socio-emotional competence in childhood. Low levels of baseline RSA in adulthood are related to psychopathology, such as emotional rigidity and poor social functioning (Butler, Wilhelm, & Gross, 2006).

In general, RSA decreases in response to a stressor. After stress RSA increases again and the body prepares for a return to homeostasis (Moore, 2009). RSA withdrawal during stress is related to positive outcomes, such as higher soothability, more attentional control, and better emotion regulation (Calkins, 1997; Huffman, Bryan, Del Carmen, Pedersen, Doussard-Roosevelt & Porges, 1998; Stifter & Corey, 2001; Suess, Porges & Plude, 1994). In addition, behavioural and emotional problems in children and adolescent are associated with less effective RSA reactivity (Beauchaine, Gatzke-Kopp, & Mead, 2007).

The effect of the SFP on infant RSA has been investigated before. In line with the polyvagal theory (Porges, 2007), the RSA of infants is higher in episodes of SFP in which they can rely on their parents (play and reunion) and is lower during the still-face episode (Bazhenova, Plonskaia, & Porges, 2001, Moore & Calkins, 2004;, Moore, Hill-Soderlund, Propper, Calkins, Mills-Koonce & Cox, 2009, Weinberg & Tronick, 1996). In addition, Ham and Tronick (2006) found that infants, who reduced their protest during the reunion episode, showed the greatest increase in RSA from the still-face episode to the reunion in comparison with children who keep protesting during the reunion.

Maternal Risk Status and the SFP

As stated before the interaction between neuropsychological vulnerability and adverse environments may have an impact on antisocial behaviour. Cumulative risk factors in the environment, such as crowding, noise, housing problems, family separation, violence, low income, single parents, and family turmoil have an effect on the reactivity of children's SNS functioning, resulting in higher physiological distress (Evans, 2003). Animal studies have shown that stress in early life may cause a reduced ability to deal with daily stressors, due to neurobiological changes (Heim & Nemeroff, 2001). Therefore, early life stress may have an impact on the physiological responses of the infants during SFP.

Previous studies already found an effect of risk status on the dyadic relationship as seen in the SFP. For a high-risk group of adolescent mothers and their infants, sensitivity is positively correlated with negative affect and negatively with self-soothing behaviour, whereas higher sensitivity was associated with less negative affect and higher levels of self-soothing behaviour for the low-risk group of middle-class adult mothers (Tarabulsy et al., 2003). Furthermore, there have been some effects of risk status on behavioural dysregulation during the play episode. Infants of mothers with high psycho-social adversity showed more dysregulation in comparison with infants of mothers with low adversities, though this effect is mediated by maternal sensitivity (Gunning, Halligan, & Murray, 2013).

Current study

The aim of this study is to examine emotion regulation of 6 months old infants during the Still-Face Paradigm (SFP). The study focuses on infant physiological responses during the SFP, namely heart rate, pre-ejection period, skin conductance and respiratory sinus arrhythmia. In addition, the physiological measures will be examined in relation to behavioural reactions to the SFP. This study will contribute to existing studies, since it will be the first to measure the pre-ejection period during the SFP. In addition, skin conductance has only been assessed in the SFP in one previous study, and to date no studies have combined all of the above-mentioned physiological indexes. There have been studies that administered the SFP to 6-month-old infants and measured behavioural reactions. However, the combination of behaviour and these autonomic nervous system indices has not been investigated before. In addition, self-soothing behaviour and arching/squirming have not been investigated thoroughly.

It was hypothesized that in response to stress (the play episode towards the still-face episode) there would be an activation of the sympathetic nervous system and inhibition of the parasympathetic nervous system. Specifically, heart rate was hypothesized to accelerate, the PEP to decrease, the SCL to increase, and the RSA to decrease. In the reunion, according to the literature, the heart rate will decelerate, PEP will increase and the RSA will increase. The effect of the SFP on SCL is unclear. Since the sympathetic nervous system will inhibit during the reunion, a decrease in SCL would be expected. However, the only study that reported SCL during the SFP reported an increase. Therefore this will be an exploratory part of the study. In addition, behavioural reactions will be associated to the physiological reactions to the SFP. The precise effects will be unclear, but is expected that more negative affect, gaze aversion and self-soothing behaviour will be associated with higher activity of indices of the sympathetic nervous system (SCL, PEP, increase in heart rate). In contrast, it is expected that more positive affect, gaze and self-soothing behaviour will be related to the higher activity of the index of the parasympathetic nervous system (RSA and decrease in heart rate).

The second aim of the study is to look for differences between the low-risk and the high-risk group in their reactions to the SFP. Previous studies have found an effect of risk status on the behavioural response of the infant during the SFP. However, the effect of risk status on physiological measures has not been investigated before. The direction of differences between high- and low-risk infants in physiological stress reactivity cannot easily be predicted from the existing literature, so these analyses are explorative. Earlier studies indicated that infants of risk mothers may show more dysregulation (Gunning, Halligan, &

Murray, 2013) and less ability to sooth themselves during stress (Tarabulsy et al, 2003). This may suggest that the infants of high-risk mothers may show more arousal, and therefore more sympathetic activity.

Methods

Background Information

The sample was drawn from a larger study: “A Good Start” which is a longitudinal study that includes 5 assessments. Mothers were interviewed at 27 weeks of pregnancy. Subsequently, the infants were assessed at 6 months of age during a home visit, at 12 months during a laboratory-session, at 20 months during a second home visit, and when they were 2.5 years old during a second lab-session. Mothers were recruited from all over The Netherlands, mostly by adverts and/or contacting maternity wards, pregnancy fairs, hospitals, and other health care facilities, although most participants came from urban agglomorations located in the Randstad. The inclusion criteria were: age of the mother, which had to be between 17 and 25 years; mothers had to be pregnant of their first child, and able to understand and speak basic Dutch. This specific age range was chosen, because in the Netherlands there is a support system for teenage mothers (aged 17 or younger), while there are only the standard facilities for mothers older than 17. The young mothers in our study are expected to be able to raise their children on their own. One of the aims of “Good start” is to study these young mothers and their children longitudinally. Exclusion criteria of the study were severe drug addiction or severe psychiatric problems, an IQ below 70 (as estimated by those entering the mothers in the study), and severe medical complications for mother or child. The study was approved by the ethics board of the Faculty of Social Science and by the medical ethical board of the Leiden University Medical Center. For the current study, the assessments at 6-months were used.

Participants

Based on the interview with mothers during the 27th week of their pregnancy, the mothers were divided into the low-risk (LR) or high-risk group (HR). Mothers were assigned to the HR group when they had multiple (≥ 2) negative factors, such as a low level of education, a poor financial situation, limited or an instabile social network, not being in a stable relationship, psychiatric problems, smoking, drinking, or drug (ab)use. The sample consists of 51 participants: 28 were assigned into the low-risk group (LR) and 23 participants were assigned to the high-risk group (HR).

Mothers from the LR group were significantly older than the mothers from the HR group, see Table 1. In addition, mothers from the HR group were more often poorly educated (χ^2 (1) = 4,79, p =.03): Most of them (83%) were only enrolled in high-school, while 46% of the LR mothers were enrolled into college. To estimate the intelligence of the mothers, 3 subtests of the Wechsler Adult Intelligence Scale-III (WAIS-III) were administered. There was no difference between the two groups on digit span backwards or matrix reasoning. However, mothers from the LR-group had significantly higher scores on vocabulary. There was no difference between the LR group and the HR group regarding the developmental functioning of the infants, as measured with the Bayley Scales of Infant Development (Van der Meulen, Ruiter, Lutje Spelberg, & Smrkovsky, 2002). Nor was there a difference in the age of the infants in months. Five infants were born preterm (less than 37 weeks), though there was no significant difference between the LR (N = 1) and HR-group (N = 4), $\chi^2(1)$ = 2.92, p =.09.

Mothers from the HR group smoked more often during pregnancy, (χ^2 (1) = 8.27, $p < .01$). There was no significant difference in the alcohol consumption (χ^2 (1) = 1.49, p = .22) and other drug-(or substance) use (χ^2 (1) = 2.74, p = .10) during pregnancy. The overall reported use of substances was low (N = 2 in HR) or absent (LR) in both groups.

Table 1

Descriptives of Background Variables

		LR		HR				
		M	*SD*	*M*	*SD*	*t*	*df*	*p*
Mothers age	years	23.68	2.25	21.64	2.74	2.90	48	<.01
Infants age	Months	5.89	.31	6.05	.58	-1.20	48	.24
WAIS III	Digitspan backwards raw	7.57	2.08	6.61	2.45	1.52	49	.14
	Vocabulary scale	9.68	3.02	7.10	3.00	2.97	47	< .01
	Matrix Reasoning scale	10.25	3.01	9.73	1.96	.74	48	.49
BSID	developmental functioning	97.04	17.67	102.60	21.55	-.97	45	.34

Procedure

Researchers went to the homes of the mothers and infants, when the infants were approximately aged 6 months (*M* = 5.96, *SD* = .45). When the infant was born preterm (less than 37 weeks), their age was corrected and they were visited a month later.

At the start of the home visit, there was some time for mother and child to adjust to the experimenters and then the electrodes were attached to the child. Subsequently, there was a baseline measure in which the child sat on the lap of the mother while they looked at a 2 minute video of Baby Einstein. Next, the Still Face Paradigm (SFP) was conducted, as designed by Tronick and colleagues (1978). The SFP consists of three consecutive 2-minute episodes. First the 'play' episode in which the mother was allowed to play with her infant, then the 'still face' episode in which the mother is asked to remain immobile and freeze her face, followed by the 'reunion' in which the mother re-engages with her infant. The infant is put into a car seat on a table and the mother sits on a chair in front of the infant facing each other. A wooden frame is placed around the infant, so that he or she is not able to look left or right. The infant was not allowed to have a pacifier. Video recordings were made to be able to code the behaviour of the infant and the mother (using a mirror that was placed above the infant on the wooden frame). After the SFP there was a recovery phase of at least 5 minutes in which the mother was allowed to pick up the infant and calm the infant down if necessary.

Measures

Behavioural measures. The behavioural reactions on the SFP were scored by two coders based upon the scoring system of Miller and Sameroff (1998). The following aspects of behavior were coded: gaze, positive affect, negative affect, self-soothing behaviour, and arching/squirming. The play episode was scored for the full 2 minutes, while the still-face and the reunion were scored for each minute separately. The still-face episode and the reunion episode were divided into 2 parts, because research has shown that there is a dynamic still-face effect in the SFP: there are changes in infant behaviour within the still-face (Ekas, Haltigan, & Messinger, 2013). Dividing the episodes into 2 parts, makes it possible to capture a change within the episode. Trained coders now watched the full fragment, while making notes, and assigned the most appropiated score for the certain fragment. A total of 20 videos (39%) were coded by both coders and an intraclass correlation was calculated.

Gaze (ICC = .83) is the amount of time that the infant gazed at the mother's face or made eye contact with mother (independent of affect). Infants were scored on a range from 0 to 3, in which a '0' represents no attention seeking; a '1' minimal attention seeking, a '2' moderate or mixed attention seeking, and a '3' predominant attention seeking. Besides gaze, the children were also scored on positive affect (ICC = .92): which is the number of smiles, that are not necessarily towards mother. On this scale the range represented intensity and frequency, in which an infant with a '0' does not smile and an infant with a '3' has several instances of positive affect or mild to moderate positive affect frequently troughout the segment. A similar system of intensity combined with frequency was used for negative affect (ICC =.91), which is the number of fusses and/or cries. Self-soothing (ICC = .90) activities include when the infants sucks on his/her body, when he/she brings an object to the mouth, when he/she sucks on hand or finger from the mother, or clasping with the hands. For self-soothing behaviour the '0' represents no engangement, '1' is minimal or low engagement, '2' is moderate or mixed engagement, and '3' is predominant or high engagement. Finally, arching/squirming (ICC = .85) is the extent to which the infant is squirming in the seat and tries to get out or arches his/her back, irrespective of gaze or affect.

Physiological measures. The Vrije Universiteit-Ambulatory Monitoring System (VUAMS) was used to measure the electrocardiogram (ECG), the impedance cardiogram (ICG), and the skin conductance (SCL). Explanations of these measures will be given underneath. Overall, seven electrodes were used: 5 ConMed Huggables REF 1620-001 electrodes for the ECG and ICG and 2 biopac EL507 electrodes for the SCL. All electrodes were connected with lead wires to the VU-AMS, which recorded the signals for the whole experiment.

The ECG is the registration of the electrical activity of the heart over a period of time. The ECG shows a typical pattern, as seen in figure 1, with a P-wave, a Q-wave, a R-peak, a S-peak and a T-wave (Van Dijk et al., 2013).

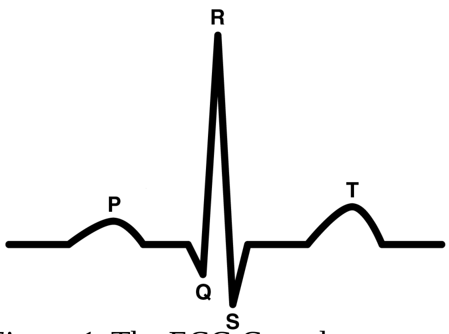

*Figure 1***.** The ECG Complex.

The ECG electrodes were placed in the following way (Van Dijk, Van Lien, Van Eijsden, Gemke, Vrijkotte, & De Geus, 2013): (1) slightly below the right collar bone to the right of the sternum, (2) on the right side between the lower ribs, and (3) on the left side slightly below the nipple. The electrodes of the ICG were placed (4) at the front at the top of the sternum (in the suprasternal notch) and (5) at the bottom of the sternum. On the back they were placed (6) on the spine a few centimetres above number 4, and (7) on the spine below number 5. The 2 SCL electrodes were placed on the soles of the feet.

After recording, the VU-AMS Data, Analysis & Management Software v2.2 software program (VU-DAMS) was used to extract the physiological indices, namely: pre-ejection period (PEP), respiratory sinus arrhythmia (RSA), and heart rate. First the data was checked manually. R-peaks, see figure 1, in the ECG were inserted if the program was not able to recognize them itself or R-peaks were deleted in the case of an artefact. An R-peak was used as an indication for a heartbeat. Suspicious beats were not deleted, because they are a natural occurrence; the heart may have skipped a beat. Next, the data was labelled, according to the accurate time registration that was done during the Still-Face Paradigm. The accuracy of the time registration is checked, by confirming the time of the control marker with the time that is registered. Subsequently, average heart rate per minute was calculated.

For impedance cardiography, 4 electrodes are used. Two electrodes induce a high frequency alternating current across the thorax, while the other electrodes receive the signals. Together they measure the change in the impedance of the enclosed thorax column (dZ), which can be used to assess the aortic blood flow (Goedhart, Willemsen, & de Geus, 2008). The impedance cardiogram (ICG) is a derivative of this change in thorax impedance using time as the basis (dZ/dt) (Riese et al, 2003). The ICG waveform is obtained by an ensemble average, which is done automatically by the software. This waveform is used to calculate the pre-ejection period (PEP), which is a measure of cardiac sympathetic activity (Newlin & Levenson, 1979).

The PEP, as shown in figure 2, is identified by calculating the interval between the Q-point in the ECG signal and the B-point in the ICG signal and. The Q-point is the onset of left ventricular depolarization (Berntson, Lozano, Chen & Cacioppo, 2004). The B-point represents the opening of the aortic valve. (Lozano et al, 2007). In response to stress, this interval will shorten and the PEP will decrease. The Q-point (ICC_{LR} = .95 and ICC_{HR} =.77) and the B-point (ICC_{LR} = .96 and ICC_{HR} =.77) are scored manually by multiple raters and a consensus score was made for all participants. Due to movement, inadequate placing of the electrodes, or due to electrodes that came loose, 17 % of all the impedance cardiograms were set as missing for the low risk group and 16% for the high-risk group.

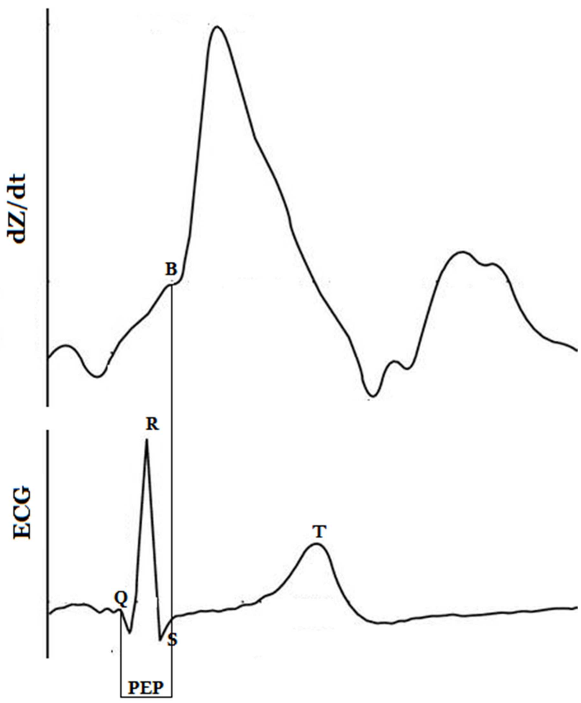

*Figure 2***.** The Pre-ejection Period.

Previous studies have shown that the Q-point can be scored as the onset of the Q-wave, however other studies have used the peak of the Q-wave, which is also the onset of the R-wave or R-onset (Berntson, Lozano, Chen, & Cacioppo, 2004). Due to movement or inadequate placing of the electrodes, the Q-wave may not be visible. An advantage of using the R-onset is that it is still possible to code even when the Q-wave is not visible. In addition, the R-onset is a more reliable way to score across scores. Therefore the R-onset was used to reflect the Q-point. Overall, 23% of the average electrocardiograms of the low-risk groups had an unclear Q-point, in comparison to 19% of the high risk. The Q-point was imputed by the average of the individual for the other episodes.

Respiratory Sinus Arrhythmia (RSA) is a measure of parasympathetic cardiac control (Bernston, Cacioppo & Quigley, 2007). The RSA was scored automatically by the software following the peak-valley method (Grossman, van Beek, & Wientjes, 1990 ; de Geus et al., 1995). First the maximum allowed deviation was set on 70%. The program then automatically detects artefacts in the respiration rate and in the IBI. Then the ECG signal is automatically compared to the respiratory signals. The program calculates a breath-tot-breath scoring and measures the shortest interbeat interval during inspiration and longest interbeat interval during expiration. Eventually a score is obtained that represents heart period variability associated with respiration (Van Dijk, et al., 2013).

Finally the Skin Conductance level (SCL) was obtained, which is considered to be a measurement of the sympathetic nervous system. The infants had 2 electrodes placed on the sole of their foot. The electrodes induce a current to the skin. Following, the conductance is reflected in electrodermal activity (Goedhart, Willemsen, & De Geus, 2008). While normally the sweat glands are used for thermo-regulation, the glands that are on the palms and on the soles of the feet are connected to emotional factors (Dawson, Schell, & Filion, 2000; Harpin & Rutter, 1982). Eventually, the average of skin conductance (in μS) is obtained automatically by the VU-DAMS program.

Statistical Analysis

The patterns of the physiological measures were examined with repeated measures analysis of variance. The pattern was first examined for the play episode into the still-face episode. This is the pattern representing stress and consists of the play episode, the first minute of the still-face episode, and the second minute of the still-face episode. The second pattern, that represents recovery, consists of the second minute of the still-face episode. the first and second minute of the reunion, and the recovery. Risk status will be included as between-group variable to assess the effect of adversities in early life on the physiological reactions during the SFP. The associations between the behavioural responses and the physiological measures were tested using Pearson's correlations.

The overall descriptives of the physiological measures during the SFP are displayed in Table 2. One HR-mother refused to continue the SFP, due to excessive crying of her infant. Therefore only baseline level, play-episode and still-face were included in the analysis of this infant. In addition, the PEP showed a high percentage of missing data. This is probably due to excessive crying, high motor activity or electrodes that came loose. Missing data often occurred for the same subject. In addition, the overall missing data percentage was rather high and missing data imputation may influence the results. Therefore the repeated-measures for PEP were executed with an *N* of 34.

In addition there was a large range of SCL during the SFP. SCL is a measurement which is easily influenced by surroundings. Temperature increase or decrease, socks on or off, type of skin, summer or winter, morning or afternoon, new or older (dryer) electrodes, etc. Experimenters tried to keep environment as standardized as possible. However, not all environmental influences could be controlled. In addition, there may be differences in the reactions of conductance of infants compared to adults. Therefore, SCL-outcomes should be interpreted with care.

There was an outlier during the baseline 1 of RSA (182.35 msec). It was decided to change the value into the second most extreme level (98.00 msec). In addition, levels of RSA during the SFP were highly skewed and had a high kurtosis. Therefore all data of the RSA were log-transformed after which the distribution was less skewed.

The descriptives of the behavioural measures are displayed in Table 3. One infant had missing scores on self-soothing behaviour due to an error in the procedure: The infant was sucking on a pacifier and was therefore not able to show self-soothing behaviour.

Table 2

Descriptives of Physiological Measures

		N	*M*	*SD*	Skewness	Kurtosis	Missing %
Heart Rate	Baseline 1	51	136.32	10.85	-.55	.36	0%
	Play	51	141.66	10.37	.07	-.52	0%
	Still face 1	51	147.07	10.01	.49	-.36	0%
	Still face 2	50	150.03	11.41	.47	.52	2%
	Reunion 1	49	148.77	12.86	1.20	3.35	4%
	Reunion 2	49	147.69	15.53	.22	.185	4%
	Recovery	50	144.44	9.25	.12	.667	2%
	Baseline 2	49	138.36	9.04	-.55	-.501	4%
PEP	Baseline 1	46	64.18	7.21	.32	1.26	10%
	Play	42	63.33	6.68	.08	-.06	18%
	Still face 1	41	63.51	7.83	-.44	.12	20%
	Still face	41	62.94	7.11	-.53	-.50	20%
	Reunion 1	38	62.42	7.45	-.33	-.33	25%
	Reunion 2	38	63.10	7.95	-.39	-.42	25%
	Recovery	44	62.43	7.31	-.70	-.44	14%
	Baseline 2	46	63.42	6.47	.03	-.04	10%
SCL	Baseline 1	51	16.74	15.33	.80	-.44	0%
	Play	51	28.31	21.57	.31	-1.27	0%
	Still face 1	51	29.47	22.01	.39	-1.02	0%
	Still face	50	30.64	21.49	.39	-.82	2%
	Reunion 1	50	32.45	21.85	.32	-.86	2%
	Reunion 2	50	33.64	22.33	.31	-.88	2%
	Recovery	50	33.07	22.03	.33	-.95	2%
	Baseline 2	50	32.74	21.22	.35	-.53	2%
RSA	Baseline 1	48	29.53	17.91	2.46	7.57	6%
	Play	48	33.94	14.54	1.30	2.37	6%
	Still face 1	49	28.35	16.54	1.47	1.70	4%
	Still face	46	27.65	18.50	2.49	8.76	10%
	Reunion 1	47	30.08	14.82	1.48	3.54	8%
	Reunion 2	47	32.79	24.66	2.04	5.49	8%
	Recovery	50	28.15	11.74	1.23	1.40	2%
	Baseline 2	47	28.76	14.91	1.52	3.33	8%
lnRSA	Baseline 1	48	3.25	.52	-.02	2.30	6%
	Play	48	3.44	.40	.19	-.12	6%
	Still face 1	49	3.20	.53	.28	-.06	4%
	Still face	46	3.16	.55	.49	.27	10%
	Reunion 1	47	3.29	.49	-.34	.67	8%
	Reunion 2	47	3.26	.67	.16	-.34	8%
	Recovery	50	3.26	.39	.20	-.01	2%
	Baseline 2	47	3.24	.50	-.09	.16	8%

Table 3

Descriptives of Behavioural Measures during the SFP

		Low Risk			High Risk			Total	
		N	*M*	*SD*	*N*	*M*	*SD*	*M*	*SD*
Positive Affect	Play	28	1.68	.91	22	1.59	1.05	1.64	.96
	Still face 1	28	.21	.42	22	.18	.50	.20	.45
	Still face 2	28	.21	.42	22	.09	.29	.16	.37
	Reunion 1	28	1.14	.85	22	1.18	.96	1.16	.89
	Reunion 2	28	1.00	.90	22	.95	1.05	.98	.96
Negative Affect	Play	28	.57	.79	22	1.09	.97	.80	.90
	Still face 1	28	.89	.88	22	1.32	1.13	1.08	1.01
	Still face 2	28	1.11	1.17	22	1.32	1.36	1.20	1.25
	Reunion 1	28	1.18	1.06	22	1.50	1.19	1.32	1.12
	Reunion 2	28	1.04	1.07	22	1.55	1.22	1.26	1.16
Gaze	Play	28	1.89	.83	22	1.45	.74	1.70	.81
	Still face 1	28	1.11	.57	22	.86	.56	1.00	.57
	Still face 2	28	1.07	.81	22	.68	.48	.90	.71
	Reunion 1	28	1.50	.84	22	1.32	.84	1.42	.84
	Reunion 2	28	1.54	1.00	22	1.23	.87	1.40	.95
Self-soothing	Play	27	1.11	1.16	21	.90	1.00	1.02	1.08
	Still face 1	28	1.04	1.26	21	.71	1.01	.90	1.16
	Still face 2	28	1.18	1.31	21	.76	.89	1.00	1.16
	Reunion 1	28	.96	1.07	21	.19	.40	.63	.93
	Reunion 2	27	1.19	1.15	21	.57	.87	.92	1.07
Arching/Squirming	Play	28	.43	.84	22	.86	.83	.62	.86
	Still face 1	28	.79	.83	22	.95	1.25	.86	1.03
	Still face 2	28	1.00	1.22	22	1.09	1.15	1.04	1.18
	Reunion 1	28	.36	.62	22	.55	.86	.44	.73
	Reunion 2	28	.39	.74	22	1.00	1.11	.66	.96

Note. Ranges are between 0 and 3.

Results

Preliminary analysis

There was no significant difference between the LR-group and the HR-group on baseline 1 of heart rate (M_{LR} = 136.00, M_{HR} = 136.72, $t(49) = -.23$, $p = .82$), on PEP baseline (M_{LR} = 65.65 , M_{HR} = 62.44, $t(44) = 1.53$, $p = .13$), on SCL baseline (M_{LR} =15.40, M_{HR} = 18.36 , $t(49) = -.68$, $p = .50$), or on RSA baseline (M_{LR} = 30.19, M_{HR} = 28.68 , $t(46) = -.29$, $p = .78$). Standard deviations are reported in Table 2.

Correlations between baselines physiological measures are displayed in Table 4. Baseline 1 was administered at the beginning of the home visit, while baseline 2 was administered in the end. All physiological measures during baseline 1 were correlated with the measure at baseline 2. In addition, heart rate and RSA were strongly negatively correlated.

Table 4

Stability and Associations of Physiological Measures

	1	2	3	4	5	6	7
1. Heart Rate baseline 1							
2. Heart Rate baseline 2	.69**						
3. SCL baseline 1	.04	.12					
4. SCL baseline 2	.06	.20	.61**				
5. PEP baseline 1	.09	.16	-.09	-.06			
6. PEP Baseline 2	.03	.14	-.10	-.11	.66**		
7. RSA baseline 1	-.64**	-.44**	.09	.11	.03	.09	
8. RSA baseline 2	-.59**	-.52**	.12	.12	-.06	-.10	.82**

Note. *$p < .05$, ** $p < .01$

The associations between the behavioural measures during the SFP are displayed in Table 5. There were correlations between the measures of the same behavioural construct over the different episodes of the SFP. Positive affect was related to all other behavioural measures: There was a negative association between positive affect and negative affect, and a positive association between positive affect and gaze. In addition, positive affect during play was related positively to self-soothing behaviour during the first minute of the still-face. Also, positive affect during the still-face was related positively to the second minute of the reunion. Positive affect during the still-face and the reunion was related negatively to arching/squirming during the still-face and the reunion.

Negative affect was positively related to arching/squirming, and more gaze during the still-face was related to more negative affect in the still-face, while more gaze in the reunion was related to less negative affect in the reunion. Gaze during the first minute of the Still-Face was positively related to self-soothing behaviour in the reunion. While gaze during the play and the reunion was related negatively to arching/squirming in the reunion.

Effect of SFP on Heart Rate

Heart rate was higher in the play-episode (M = 141.66, SD = 10.37), compared to the baseline (M =136.32, SD = 10.85), $t(50) = -4.66$, p < 01. During the play and the still-face episode, there was no difference between the LR-group and HR-group on heart rate, $F(1,48)$ = .01, p =.94. Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of episode, $\chi^2(2) = 29.36$, $p < .01$, and multivariate tests will be reported ($\varepsilon = .69$). There was a significant effect of episode on heart rate, V= .34, $F(2,48) = 12.48$, p <.01, $\eta^2 = .58$. The still-face episode was associated with an increase in heart rate, see Figure 3. There was no interaction-effect for episode and risk status, V = .02, $F(2,47) = .02$, p = .65.

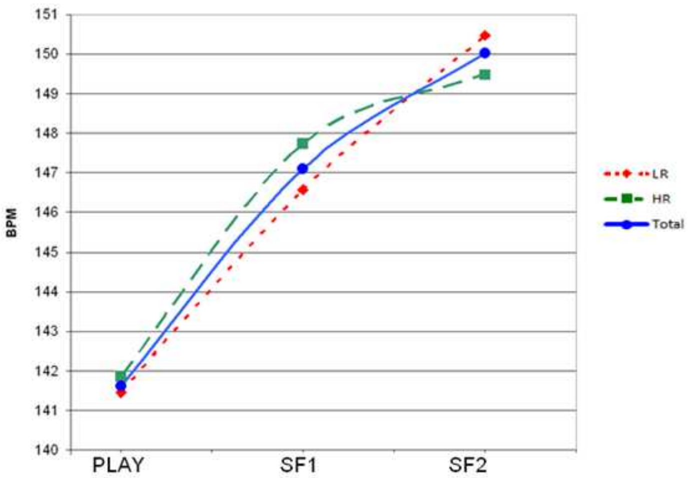

*Figure 3***.** Influence of still-face on heart rate.

Table 5

Correlations between Behavioural Measures

	PA.play	PA.sf1	PA.af2	PA.re1	PA.re2	NA,play	NA.sf1	NA.sf2	NA.re1	NAre2	GZ.play	GZ.sf1	GZ.sf2	GZ.re1	GZ.re2	SS.play	SS.sf1	SS.sf2	SS.re1	SS.re2	AS.play	AS.sf1	AS.sf2	AS.re1
PosAff Sf1	.26																							
PosAff Sf2	**.28***	.17																						
PosAff Re1	**.35***	.17	**.36***																					
PosAff Re2	**.48****	.20	.18	**.63****																				
NegAff play	**-.44****	-.05	-.21	-.19	-.22																			
NegAff Sf1	-.22	-.08	**-.31***	**-.38****	**-.36***	**.67****																		
NegAff Sf2	.15	.15	**-.38****	**-.31***	-.13	**.35***	**.70****																	
NegAff Re1	-.08	.03	-.28	**-.55****	**-.45****	**.39****	**.65****	**.67****																
NegAff Re2	-.21	-.14	-.24	**-.44****	**-.55****	**.44****	**.61****	**.46****	**.77****															
Gaze play	**.43****	.06	.10	.10	.25	-.08	.03	.10	.06	-.02														
Gaze Sf1	.22	**.32***	.10	**.28***	.26	.12	.11	**.29***	.06	-.06	**.35***													
Gaze Sf2	.07	-.13	.14	.19	.03	.10	.07	.09	.02	.01	.20	**.56****												
Gaze Re1	**.37****	-.01	.11	**.49****	**.44****	.03	-.09	.11	-.10	-.12	**.28***	**.60****	**.49****											
Gaze Re2	**.36****	.01	.05	**.33***	**.59****	.02	-.10	.17	-.20	**-.34***	**.40****	**.49****	**.34***	**.69****										
SSooth play	.11	-.01	.15	-.01	-.02	-.11	-.14	-.07	.07	-.04	-.02	-.14	-.11	-.06	-.07									
SSooth Sf1	**.35***	.09	.09	.08	.13	-.24	-.06	-.02	-.01	.02	.12	.09	-.04	.13	-.01	**.37****								
SSooth Sf2	.20	.05	.19	.24	.19	-.24	-.21	-.15	-.16	-.08	-.07	-.03	.13	.22	.01	.24	**.59****							
SSooth Re1	.22	.12	.18	.05	.04	-.25	-.23	-.01	-.01	-.12	.18	**.31***	.16	.17	.07	**.35***	**.49****	**.35***						
SSooth Re2	.13	**.35***	.14	.04	.08	-.25	-.11	-.05	-.09	-.15	.11	.17	.10	.02	-.07	.09	**.39****	**.50****	**.65****					
Arching play	-.07	-.06	-.13	-.19	-.21	.27	**.32***	.27	.28*	.27	-.17	-.13	-.23	-.14	-.16	.12	.01	-.13	-.10	**-.31***				
Arching Sf1	.09	-.03	-.26	-.22	-.25	.19	**.44****	**.55****	**.50****	**.43****	.17	.10	.06	-.01	.02	-.11	-.10	-.24	.03	-.11	**.50****			
Arching Sf2	.18	.22	**-.30***	-.20	-.18	.08	**.31***	**.52****	**.43****	.20	.18	.27	-.04	-.02	.06	-.07	-.13	-.25	.14	.01	**.38****	**.69****		
Arching Re1	-.06	.22	-.19	**-.42****	**-.39****	.23	**.37****	**.35***	**.60****	**.49****	-.01	-.15	-.23	-.28	-.26	.20	.15	-.12	.08	.02	**.40****	.27	**.29***	
Arching Re2	-.22	.02	-.19	-.10	**-.36****	.27	**.49****	**.31***	**.43****	**.58****	**-.34***	-.15	-.20	-.17	**-.43****	-.11	-.01	.01	-.24	-.15	**.54****	**.49****	**.28***	**.33***

PA= Positive Affect
NA= Negative Affect
GZ= Gaze
SS = Self-soothing
AS =Arching/Squirming

Sf1 = First minute Still-face episode
Sf2 = Second minute Still-face episode
Re1 = First minute Reunion episode
Re2 = Second minute Reunion episode

Note. Bold script represents significant relations, $*p < .05$, $** p < .01$

The recovery of stress is shown in Figure 4. There was no sphericity as indicated by the Mauchly's test, $\chi^2(5) = 27.88$, $p < .01$, ($\varepsilon = .77$). The multivariate tests indicated that there was an effect of episode on the heart rate, $V = .25$, $F(3,46) = 5.00$, $p < .01$, $\eta^2 = .25$. After the still-face the heart rate decreased during the reunion and decreased furthermore in the recovery. In addition, there was no interaction effect of episode and risk status, $V = .11$, $F(3,45) = 1.89$, $p = .15$, nor was there a main effect of risk status, $F(1,47) = .07$, $p = .79$.

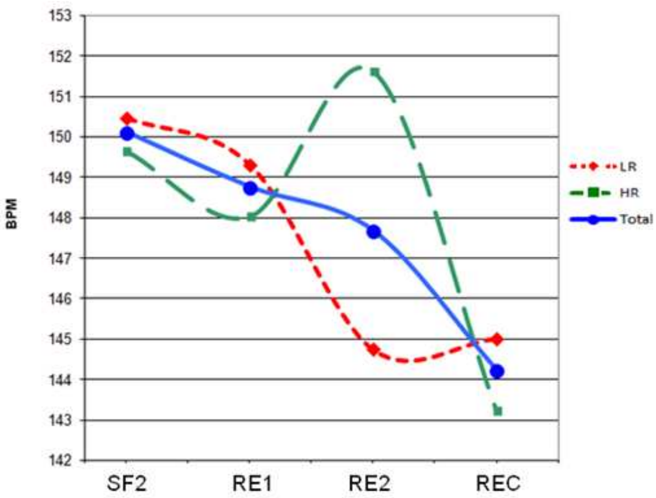

Figure 4. Recovery of heart rate

Figure 4 does however indicate a possible difference in the recovery patterns of the two groups. Therefore, this difference was investigated in more detail. The assumption of sphericity was met, $\chi^2(2) = 2.12$, $p = .35$. There was no main effect of risk status, $F(1,47) = .18$, $p = .67$. However, there was a significant interaction-effect of risk status and episode on heart rate, $F(2,94) = 3.19$, $p = .05$, $\eta^2 = .07$. Thus, infants from the HR-group had a similar heart rate compared to the LR group, though both groups reacted differently to the SFP. The heart rate of HR-infants increased during the reunion and decreased during the recovery, while the heart rate of LR-infants already decreased during the reunion.

Paired t-test indicated that the heart rate did not restore in the recovery ($M = 144.44$, $SD = 9.25$) to baseline 1 level ($M = 136.32$, $SD = 10.85$, $t(49) = -6.48$, $p < .01$), nor to the level at the play-episode ($M = 141.66$, $SD = 10.37$, $t(49) = -2.49$, $p = .02$).

Effect of SFP on Pre-ejection Period

There was no difference in the PEP between the baseline (*M* = 64.18, *SD* = 7.21) and the play-episode (*M* = 63.33, *SD* = 6.68), *t*(38) = .22, *p* = .82. The assumption of sphericity was met, $\chi^2(2) = .94$, *p* = .63. There was no effect of the Still-face episode on the PEP, *F*(2,66) = .13, *p* =.88. Neither was there a significant effect of risk status on the PEP in the play and still face episode, *F*(1, 32) = .97, *p* = .33. Nor was there an interaction-effect of episode and risk status on heart rate, *F*(2, 64) = 2.23, *p* =.12. The overall patterns are presented in Figure 5.

Next, the recovery pattern was investigated (Figure 6). The assumption of sphericity was met, $\chi^2(5) = 5.83$, *p* = .32. There was no effect of episode on the PEP, *F*(3,93) = .56, *p* = 65. Neither was there a significant effect of risk status on the PEP, *F*(1,30) = 1.71, *p* = .20, nor an interaction effect of risk status and episode, *F*(3,90) = .84, *p* =.48.

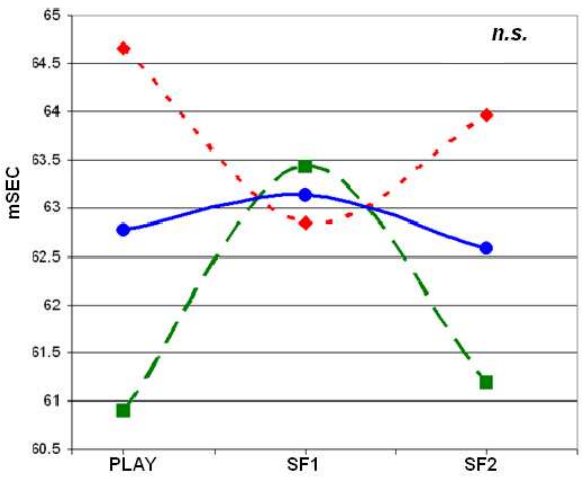

*Figure 5***.** Influence of still-face on PEP**.**

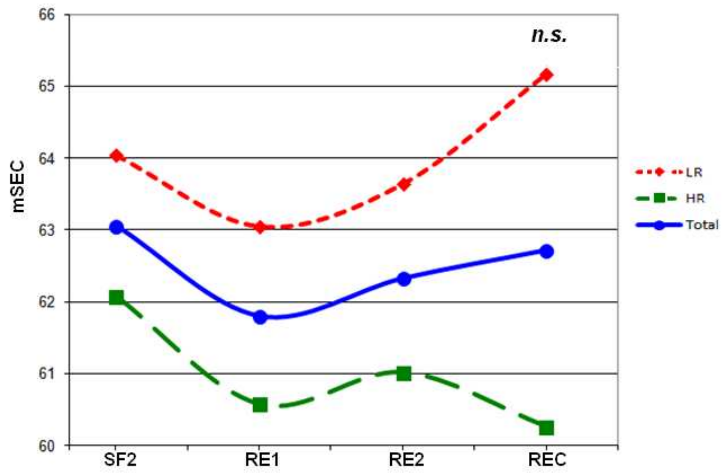

Figure 6. Recovery of PEP

Effect of SFP on Skin Conductance

There was a significant difference between SCL level at the baseline (*M* = 16.74, *SD* = 15.33) and SCL level at the play-episode (*M* = 28.31, *SD* = 21.57), *t*(50) = -6.56, *p* <.01. First the effect of the stressful episodes will be discussed: Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(2) = 29.01$, *p* < .01. Multivariate tests (ε = .69) indicate a significant effect of the play and still-face episode on SCL, *V* =.13, *F*(2,48) = 3.69, *p* = .03, η^2 =.13. The still-face episode was related to an increase in SCL, see Figure 7. There was no effect of risk status,

$F(1,48) = .62$, $p = .43$. Nor was there an interaction-effect between risk status and episode on SCL, $V = .01$, $F(2,47) = .27$, $p = .76$.

In regard to the recovery pattern, the assumption of sphericity was violated as well. $\chi^2(5) = 50.64$, $p < .01$. Therefore a multivariate test ($\varepsilon = .70$) was used. There was a significant effect of episode on SCL, $V = .20$, $F(3, 47) = 3.97$, $p < .01$, $\eta^2 = .20$. Overall, there was an increase in SCL over the episodes and a slight decrease during the reunion. In addition there was a significant interaction-effect between risk status and episode, $V = .23$, $F(3, 46) = 4.52$, $p < .01$, $\eta^2 = .23$. There was no main effect of risk status on SCL, $F(1, 48) = 1.46$, $p = 23$. So there was no difference between the groups on SCL, however they did show different patterns over the episodes. The infants from the HR-group showed a higher increase over the reunion episodes and a decrease in the recovery see Figure 8.

Overall, the SCL did not restore in the recovery ($M = 33.07$, SD = 22.03) to baseline 1 ($M = 16.74$, $SD = 15.33$, $t(49) = -8.29$, $p < .01$) or to the level of the play-episode ($M = 28.31$, $SD = 21.57$, $t(49) = -5.37$, $p < .01$.)

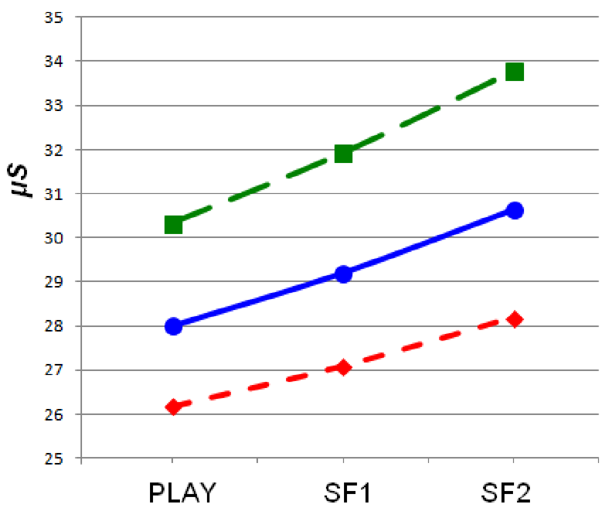

Figure 7. Effect of Still-Face on SCL.

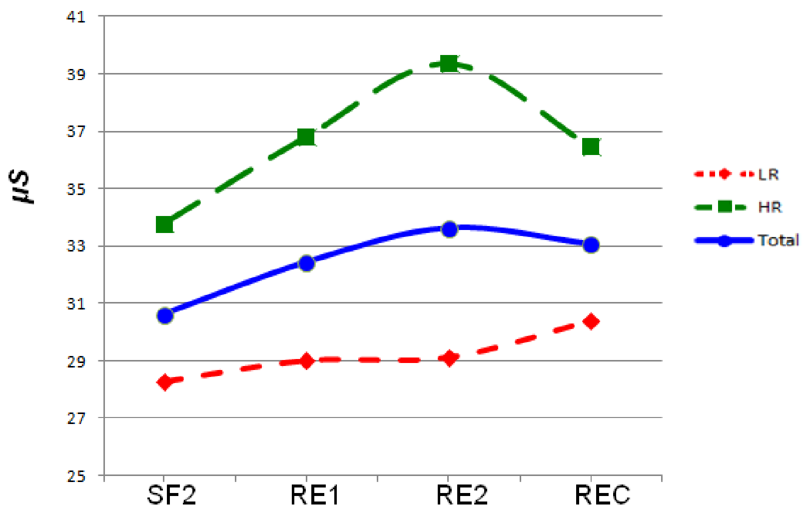

Figure 8. Recovery of SCL

There was a significant difference between the baseline level lnRSA ($M = 3.25$, $SD = .52$) and lnRSA level during the play-episode ($M = 3.44$, $SD = .40$), $t(44) = -2{,}64$, $p = .01$. Furthermore, the effect of the play episode and the still-face episode on lnRSA is examined: The assumption of sphericity was met, $\chi^2(2) = 2{,}84$, $p = .24$. There was a significant effect of episode on lnRSA, $F(2,84) = 5.15$, $p <.01$, $\eta^2 =.15$ and there was no main effect of risk status on lnRSA, $F(1,41) = .83$, p = .37. Besides, there was no significant interaction effect, $F(2,82) = .83$, $p = .44$. Overall, both groups showed a similar decrease in lnRSA over the first episodes of the SFP, see Figure 9.

The assumption of sphericity was not met for the episodes in the recovery pattern, $\chi^2(5) = 42.25$, $p < .01$. Multivariate tests ($\varepsilon = .66$) indicated that there was no effect of episode on lnRSA, $V =.08$, $F(3,42) = 1.20$, $p = .32$ (see Figure 10), nor was there a main effect of risk status, $F(1,43) = .05$, $p = .83$, or an interaction effect of risk status and episode, $V =.01$, $F(3,41) = .14$, $p = .94$.

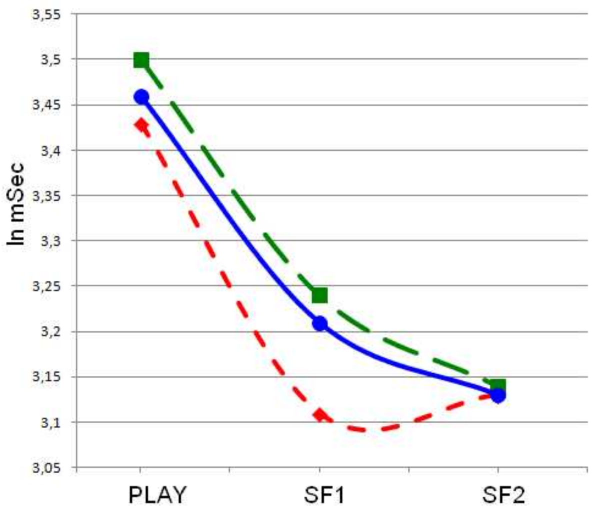

Figure 9. Effect of Still-Face on lnRSA.

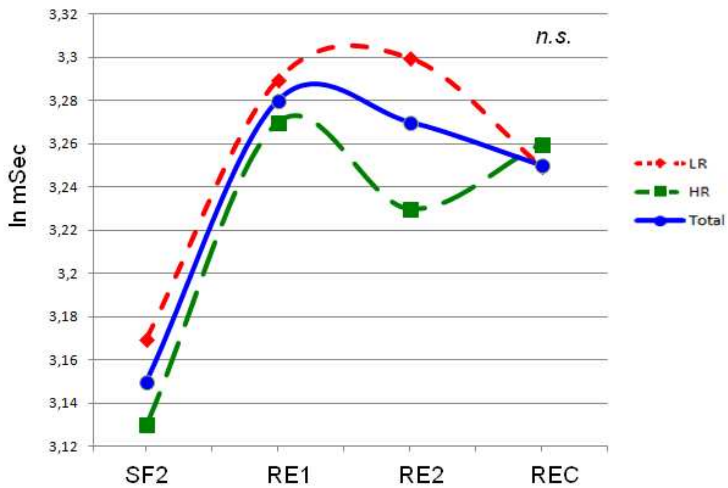

Figure 10. Recovery of lnRSA.

Associations between Physiological Measures

The associations between the physiological measures during the SFP are displayed in Table 6. There were significant negative relations between heart rate and RSA. If the heart rate went down, the RSA went up and the other way around. These relations were strong. SCL was not related to the other physiological measures. In addition, there were some significant negative relations between heart rate and PEP. The relations were moderate to strong. In addition, there were some positive relations RSA and PEP. If the RSA went up, the PEP went up as well. The relations were moderate to strong as well.

Associations Physiological Measures with Behaviour

Tables 7 and 8 indicate that there were associations between heart rate and positive affect, negative affect and arching/squirming. Due to the high amount of correlations, careful interpretation of the results is required. When the infant showed more positive affect, the heart rate was lower. In addition, when the infant showed more negative affect the heart rate was higher. This was also the case for arching and squirming. Furthermore, there were negative associations between PEP during still-face/reunion and positive affect during the play, PEP during still-face/reunion and negative affect during the still-face, and PEP during the play and arching/squirming in other episodes. In addition there is a positive association between PEP during still-face/reunion and self-soothing behaviour during play and during the first minute of the still-face. This last association is a very strong relation, r =.92. Overall, the PEP was higher when the infants showed more self-soothing behaviour and less arching/squirming. In addition, more positive affect and more negative affect were associated with a smaller PEP.

Only one association of SCL with behavioural measures was significant: There was a positive moderate association between gaze in the first minute of the still-face and SCL during the second minute of the still-face. In addition, it should be noted that this is also the only association of gaze with the physiological measures.

However there were associations, during certain episodes, between the lnRSA and positive affect, negative affect, self-soothing behaviour and arching/squirming. The lnRSA was related positively to positive affect during the play and during the first minute of the still-face. However, it was negatively related in the second minute of the still-face. LnRSA during still-face/reunion was related negatively to negative affect during still-face and reunion. Self-soothing behaviour during still-face and reunion was positively associated with lnRSA during the reunion. Arching/squirming during play and still-face was negatively related with lnRSA during the reunion. All significant associations of lnRSA with behaviour are of moderate strength. Overall, the lnRSA was high when infants showed more positive affect and more self-soothing behaviour. The lnRSA was low when infants showed more negative affect and more arching/squriming.

Table 6

Correlations between Physiological Measures during the SFP

	HRT play	HRT sf1	HRT sf2	HRT re1	HRT re2	HRT rec1	PEP play	PEP sf1	PEP sf2	PEP re1	PEP re2	PEP rec	SCL play	SCL sf1	SCL sf2	SCL re1	SCL re2	SCL rec	RSA play	RSA sf1	RSA sf2	RSA re1	RSA re2
HRT play																							
HRT sf1	**.71****																						
HRT sf2	.20	**.59****																					
HRT re1	.25	**.56****	**.76****																				
HRT re2	**.38****	**.43****	**.32***	**.54****																			
HRT rec1	**.68****	**.77****	**.48****	**.47****	**.36***																		
PEP play	.18	-.06	**-.38***	-.22	-.17	-.16																	
PEP sf1	.14	-.02	-.19	-.15	-.09	-.12	**.64****																
PEP sf2	.21	-.23	**-.47****	**-.45****	-.17	-.16	**.63****	**.60****															
PEP re1	.19	-.08	-.31	-.19	-.02	-.14	**.76****	**.71****	**.73****														
PEP re2	-.02	-.32	**-.39***	**-.44****	-.26	**-.37***	**.74****	**.79****	**.81****	**.71****													
PEP rec	**.31***	-.07	-.26	-.13	-.10	.01	**.70****	**.64****	**.70****	**.72****	**.65****												
SCL play	.17	.19	.05	-.03	.01	.04	-.13	-.16	-.24	-.25	-.24	-.23											
SCL sf1	.13	.18	.05	-.01	-.01	.01	.19	.17	.11	.10	.09	.13	**.98****										
SCL sf2	.04	.13	.09	.01	.01	-.04	-.15	-.17	-.23	-.25	-.22	-.24	**.95****	**.98****									
SCL re1	.01	.11	.08	.01	.03	-.05	.01	-.01	-.09	-.10	-.09	-.10	**.94****	**.96****	**.99****								
SCL re2	.01	.10	.06	-.02	.07	-.04	-.06	-.08	-.14	-.17	-.15	-.18	**.93****	**.94****	**.96****	**.99****							
SCL rec	.01	.12	.07	-.04	.03	.00	-.06	-.08	-.16	-.19	-.21	-.20	**.95****	**.95****	**.95****	**.97****	**.97****						
RSA play	-.47	**-.29***	.02	.06	-.18	**-.29***	-.03	.07	.30	.22	-.01	.07	-.12	-.09	-.05	-.01	-.01	-.03					
RSA sf1	**-.36***	**-.41****	-.09	-.22	**-.38****	**-.39****	.13	.08	.07	.26	.12	.15	.02	.01	.01	.05	.08	.06	**.44****				
RSA sf2	.00	-.17	**-.45****	**-.53****	-.24	-.15	-.16	-.09	.12	**.35***	.01	-.02	.28	.26	.21	.24	.25	.28	.06	**.37***			
RSA re1	-.17	**-.33***	**-.39****	**-.58****	**-.42****	-.24	.02	.11	.23	**.41***	.07	.14	.18	.15	.07	.11	.17	.18	**.31***	**.44****	**.47****		
RSA re2	**-.34***	-.29	-.05	-.22	**-.64****	**-.32***	.00	.09	.15	**.41***	.12	.20	.20	.20	.16	.17	.17	.19	**.58****	**.68****	**.34***	**.57****	
RSA rec	**-.43****	**-.29***	-.06	-.27	**-.39****	**-.36***	-.13	-.05	.11	.18	-.02	-.01	.14	.14	.12	.16	.18	.19	**.55****	**.66****	**.46****	**.56****	**.69****

Sf 1 = first minute of still face
Sf 2 = second minute of still face
Re1 = first minute of reunion
Re2 = second minute of reunion

Note. Bold script represents significant relations, $^{*}p < .05$, $^{**}p < .01$

Table 7

Correlations Physiological Measures and Infant Affect During the SFP.

		Positive affect					Negative Affect				
		Play	Still face 1	Still face 2	Reunion 1	Reunion2	Play	Still face 1	Still face 2	Reunion 1	Reunion2
Heart Rate	Play	**-.39****	-.25	-.03	-.04	.07	.27	.05	-.13	-.15	-.09
	Still Face 1	-.13	-.19	-.10	-.10	.07	**.30***	.33*	.27	.13	.07
	Still Face 2	**.31***	.08	-.17	-.23	.03	.01	**.32***	**.61****	**.34***	.17
	Reunion 1	.13	.08	-.18	**-.41****	-.11	.09	**.41****	**.55****	**.60****	**.37****
	Reunion 2	-.16	**-.29***	-.11	-.25	**-.32***	.22	**.32***	.15	**.43****	**.62****
	Recovery	-.20	**-.33***	-.03	-.08	.02	.13	.13	.02	-.03	.03
PEP	Play	-.25	-.03	-.04	-.01	.05	-.03	-.21	**-.32***	-.23	-.22
	Still Face 1	-.27	.12	-.14	-.10	-.01	.11	-.12	-.05	-.05	-.01
	Still Face 2	**-.42****	-.28	.10	-.03	.02	.10	**-.38***	**-.41****	-.31	-.18
	Reunion 1	**-.45****	-.19	-.22	-.24	-.20	.25	.04	-.16	-.02	.02
	Reunion 2	-.09	.03	-.05	-.02	.04	-.03	-.20	-.14	-.16	-.03
	Recovery	**-.30***	-.17	.03	-.17	-.04	.05	-.14	-.26	-.10	-.10
SCL	Play	-.11	.08	-.13	.05	.03	-.02	.07	.11	-.05	-.04
	Still Face 1	-.09	.09	-.12	.08	.04	.01	.12	.14	-.03	-.04
	Still Face 2	-.03	.09	-.09	.06	.02	-.02	.16	.22	.03	-.01
	Reunion 1	-.02	.07	-.08	.04	.02	-.03	.18	.23	.03	.02
	Reunion 2	-.05	.05	-.07	.03	.01	-.04	.15	.18	.01	.05
	Recovery	-.06	.05	-.05	.06	-.01	-.08	.09	.13	-.05	-.02
lnRSA	Play	**.31***	**.35***	.05	-.03	.01	.06	.13	.18	**.29***	.12
	Still Face 1	.03	.23	**-.35***	-.12	-.08	-.03	-.01	.09	.07	.04
	Still Face 2	-.13	-.22	.02	.23	.02	.11	-.10	-.27	**-.33***	-.16
	Reunion 1	-.13	.01	.06	.13	.12	.01	-.17	**-.29***	**-.32***	-.14
	Reunion 2	.27	**.29***	-.06	.05	.22	-.05	-.11	.10	-.01	-.24
	Recovery	.12	.20	-.10	.02	-.04	-.01	.10	.21	.08	.07

Note. Bold script represents significant relations, $^{*}p < .05$, $^{**}p < .01$

Table 8

Correlations between Physiological Measures and Infant Gaze, Self-soothing Behaviour, and Arching/Squirming.

		Gaze					Self-soothing behaviour					Arching/Squirming				
		Play	Still face 1	Still face 2	Reunion 1	Reunion2	Play	Still face 1	Still face 2	Reunion 1	Reunion2	Play	Still face 1	Still face 2	Reunion 1	Reunion2
Heart Rate	Play	-.17	-.08	.02	-.08	.09	.11	-.10	-.11	-.09	-.11	.19	-.25	-.08	-.06	-.06
	Still Face 1	.06	-.04	.05	-.07	.18	-.12	-.18	-.10	-.19	-.05	.19	.02	.11	-.04	.13
	Still Face 2	.25	.09	-.07	-.01	.19	-.07	.01	-.08	.16	.18	.17	**.36****	**.55****	.18	.09
	Reunion 1	.24	.11	-.03	-.06	.03	-.05	.05	-.13	.19	.10	.22	**.31***	**.38****	**.37****	.18
	Reunion 2	-.03	-.09	.01	-.08	-.15	.17	-.02	-.11	-.02	-.15	.12	.18	.13	.14	**.32***
	Recovery	-.05	-.19	-.03	-.16	-.02	-.15	-.06	-.01	.04	.05	.17	-.07	-.05	.03	.11
PEP	Play	.03	.01	.08	-.01	.16	.15	.03	.02	-.01	-.01	**-.41****	**-.43****	**-.38***	-.23	**-.33***
	Still Face 1	-.22	.04	.03	.04	.03	-.02	.11	**.92***	.08	.17	.01	-.07	.04	-.21	.05
	Still Face 2	-.16	-.12	.09	.13	.07	**.32***	-.02	.27	.12	-.10	-.07	-.30	**-.34***	-.29	-.24
	Reunion 1	.01	.03	.09	-.04	.02	**.34***	.15	.19	.18	.14	.02	-.09	-.11	-.01	-.02
	Reunion 2	.01	.01	.15	.12	.05	.25	.27	.35*	.01	.02	-.21	-.27	-.30	-.25	-.20
	Recovery	-.01	.05	.15	.15	.14	.14	.13	.20	.11	.02	-.08	-.17	-.14	-.14	-.13
SCL	Play	-.05	.20	.05	.04	.12	-.10	-.07	.02	-.18	.06	.07	-.03	.23	-.05	.04
	Still Face 1	.01	.25	.08	.07	.14	-.11	-.06	.04	-.20	.03	.07	.01	.24	-.06	.05
	Still Face 2	.04	**.29***	.09	.08	.14	-.08	-.04	.01	-.17	.01	.11	.11	**.30***	-.06	.08
	Reunion 1	-.02	.24	.07	.06	.12	-.09	-.06	.01	-.19	-.01	.09	.11	.27	-.05	.11
	Reunion 2	-.06	.15	.01	.02	.08	-.06	-.07	.03	-.22	.01	.06	.06	.20	-.03	.12
	Recovery	-.07	.14	.04	-.01	.06	-.15	-.12	.03	-.19	.07	.01	.05	.22	-.08	.09
lnRSA	Play	.06	.05	-.02	-.11	-.10	.12	.17	.18	.27	.29	-.05	.03	.08	.22	.05
	Still Face 1	-.01	-.01	-.07	-.18	-.11	.01	-.04	-.07	.02	.16	-.12	.14	.20	**.31***	-.08
	Still Face 2	-.07	.11	.09	.07	.08	.10	-.15	-.13	-.10	-.11	-.19	-.26	-.22	-.18	-.23
	Reunion 1	-.11	-.07	.09	-.03	.03	.12	.22	**.42****	.04	**.31***	**-.31***	**-.37***	**-.31***	-.03	-.10
	Reunion 2	.19	.14	.02	.01	.18	.01	.10	.21	.13	.26	-.16	-.14	.11	.21	**-.33***
	Recovery	-.05	.06	-.15	-.06	-.04	.06	.08	.07	-.01	.16	-.09	.01	.05	.12	.11

Note. Bold script represents significant relations, $^{*}p < .05$, $^{**}p < .01$

Discussion

Earlier studies have suggested that emotion regulation is one of the precursors of antisocial behaviour in later life (Calkins & Dedmon, 2000; Gilliom, Shaw, Beck, Schonberg & Lukon). The aim of the present study was to examine infant emotion regulation by measuring changes in the autonomic nervous system during the Still-Face Paradigm (SFP). This is the first study that measures the effect of the SFP on the pre-ejection period (PEP) of the infant. In addition, it is the first study that investigated the effect of the SFP on heart rate, PEP, respiratory sinus arrhythmia (RSA), and skin conductance (SCL) together. It was hypothesized that during the Still-face episode, the sympathetic nervous system (PEP, SCL, increase heart rate) would activate and the parasympathetic nervous system would inhibit (RSA, decrease heart rate) and these effects would be reversed for the play and reunion episode. The second aim of this study was to examine the physiological changes in relation to the risk status of the infant. The third aim was examine the associations between behavioural reactions to the SFP to the physiological reactions.

General Physiological Effects of the SFP

Overall the SFP was able to elicit a sympathetic and a parasympathetic response. In response to the still-face episode the sympathetic activity increased and the parasympathetic activity decreased. The physiological reactions during the reunion are varying and will be discussed later on.

Consistent with the hypothesis, an effect of the SFP on heart rate was found. During the transition from the play to the still-face episode, the heart rate increased significantly. The disconnection of interaction with mother was associated with an increase in heart rate. In addition, there was a significant decrease during the transition from the still-face to the reunion episode. These effects indicate that the still-face can be seen as stressful to the infant. In addition, these findings provide physiological support for the classic still-face effect, in which children typically show stressful behaviour during the still-face as a result of prolonged and intense mismatches in social communication between mother and infant (Mesman et al., 2009).

Sympathetic nervous system. There was no effect of the SFP on infant PEP, which indicates an absence of sympathetic activity. However, due to missing data the number of infants that were included was rather low (*N*=34). It is possible that the remaining sample was too small to find significant effects. It is also possible that the SFP was not stressful enough to elicit a strong reaction of the sympathetic nervous system. On the other hand, there was an effect of SFP on SCL. The SCL, which is also a measure of sympathetic activity, increased during the SFP, and increased again during the reunion and started to descend during the recovery. This is in line with the only study that investigated SCL during the SFP (Ham and Tronick, 2006). This may indicate that SCL needs a longer time to recover from the still-face.

Thus an increase in sympathetic activity was found with respect to skin conductance, but not for the PEP. Earlier studies did find that SCL and PEP were associated with the activity of the sympathetic nervous system (De Geus & Van Doornen, 1996; Dawson, Schell, & Filion, 2000). However, Goedhart and colleagues (2008) investigated both systems and they did not find a correlation between SCL and PEP. This lack of correlation is also found in this study. Goedhart and colleagues (2008) explored this absence of correlation between PEP and SCL and found an absence of correlation between subjects and an absence within the individual. The absence of an association between subjects could be explained by individual differences in the responsiveness of the systems, such as the percentage of sweat ducts per area of skin. In addition, the absence of an association within subjects may reflect a difference in the activation of SCL and PEP which are both indices of the sympathetic nervous system. It is possible that other mechanisms also play a role in the activation of the sympathetic nervous system. For example the effect of the baroreflex. This is a mechanism that is responsible for the maintenance of blood pressure. The baroreflex does have an influence on sympathetic cardiac activity, but not on skin activity. Therefore, there can be an increase in activity in one, but not in the other, which may explain the absence of effect of PEP and the existence of an effect of SCL.

Parasympathetic nervous system. An inhibition of the parasympathetic nervous system was found during the transition from the play to the still-face episode: the lnRSA decreased. However, no effect was found after the changeover to the reunion. This may be an indication that the parasympathetic nervous system was still inhibited. Infants may still be influenced by the still-face, due to the so-called carry-over effect. This effect has been described previously for behavioural measures: After the still-face positive affect increases, but does not return to the level at play. In addition, negative affect decreases, but the level does not go back to the level at play (Mesman et al., 2009). This may indicate that the infants still feel challenged, which prevents them from activating their parasympathetic nervous system to be able to rest and digest. This carry-over effect is also supported by the level of heart rate and SCL during the reunion. The heart rate decreased during the reunion, but did not restore to the level at play. In addition, the SCL increased furthermore in the reunion, both indicating that the infants were negatively affected by the still-face.

Associations between Physiological and Behavioural Responses to the SFP

The SFP is able to elicit a physiological response, but it also evokes a behavioural response. Earlier studies have stated that it is possible to regulate emotions by controlling one's behaviour (Ochsner & Gross, 2005). This study indicated that there are associations between the behavioural reactions and the physiological reactions during the SFP. More positive affect, less negative affect, and less arching/squirming was related to more parasympathetic activity and less sympathetic activity, while the opposite of these behaviours (less positive affect, more negative affect, and more arching/squirming) was related to more sympathetic activity. Gaze was not related to any physiological measure, except for one negligible relation with SCL. This indicates that the amount of gaze may not be related to either sympathetic or parasympathetic activity. Therefore gaze (as in looking away) may not be a suitable strategy to control physiological arousal. In addition, no behavioural measures were related to SCL, except for the one with gaze. This points to an inability to control electrodermal activity by behaviour in infants.

Self-soothing behaviour was related strongly and positively to the PEP. In addition, self-soothing behaviour is related positively to lnRSA. This may indicate that during self-soothing behaviour the infants shows more parasympathetic activity and that self-soothing behaviour is related to calming down. This is in line with previous studies that state that self-soothing behaviour is a strategy to cope with stress (Stifter & Braungart, 1995).

HR-LR Differences regarding Physiological Effects

Infants from the HR-showed atypical patterns in heart rate and SCL during the reunion and recovery. Their heart rate increased during the reunion, while the heart rate of the LR-group decreased. In addition, the SCL increased faster and higher for the HR-group in the reunion, while the LR-group showed a flat pattern of SCL. In both cases, the infants from the HR-group showed an higher stress reaction. They did not calm down when the mother tried to reunite, but showed an even greater stress reaction. In contrast, the LR-group did calm down. These results suggest that the infants from a high risk sample were less able to regulate their emotions

Earlier studies have found that higher sympathetic activity during middle childhood was related to more externalizing behaviour (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008). A meta-analysis by Lorber (2004) indicated that more electrodermal activity (such as skin conductance) is related to aggression. In addition high heart rate reactivity was also related to aggression and conduct problems. These effects were found for children as well as adults. All in all, these studies indicate that the higher sympathetic activity of the HR-group in this study may put them at risk for developing externalizing behaviour problems.

A possible cause of these differences between the HR-group and LR-group is the difference in surroundings in which the infants grows up. The HR mothers were selected on characteristics that may have an adverse on the infants development. So in comparison to the LR-group, infants from the HR-group had mothers, which were on average less well educated, financially worse off, had a more limited or less stable social network, were more often without or with varying partners, showed higher prevalence of psychiatric problems, and smoked, drank or (ab)used drugs more often. A meta-analysis indicated that children living under high-risk conditions are more likely to develop insecure and disorganized attachment patterns in comparison with children living in low-risk families (Cyr Euser, Bakermans-Kranenburg, & Van IJzendoorn, 2010). In addition, the risk for attachment disorganization is higher when children are exposed to multiple socioeconomic risks. It is possible that the HR-mothers in this study were less effective in soothing their babies. They may be less sensitive or responsive to the needs of their babies. Another possibility is that they showed frightening or frightened behaviour during the reunion. This could result in a stress reaction during the reunion, which may explain the increase in heart rate and SCL.

Limitations and Future Directions

The current study had a high percentage of missing data, namely 33% for the PEP. Especially the part that is due to excessive crying and moving could be a problem, because this may present a group who is not able to regulate their emotions and who would score high on the indices of sympathetic activity. Movement disrupts the signal of the ICG and therefore makes it impossible to score the PEP. As a result the effect of the SFP on the PEP, may be underestimated. Though, this was the first study that included the PEP during the SFP. The study should be replicated with a larger sample; this may lead to more realistic results.

In addition, the overall range of SCL was large: the mean ranged between 16 and 33 μS and the standard deviation between 15 and 22 μS. An earlier study investigated skin conductance of infants during a stress task and a mean of 16.80 μS and a standard deviation of 11.71 μS was reported (Baker, Shelton, Baibazarova, Hay, & Van Goozen, 2013). Studies that use skin conductance at the intensive care to assess the emotional state of the newborn babies also report smaller ranges in SCL: A study that assessed infant skin conductance during sleep, awake moments, and during crying and a mean skin conductance level was reported that ranged between 0 and 10 μS (Storm, 2001). Considering the lack of data regarding skin conductance activity during the SFP from previous studies, the results from the present study should be considered a first step towards understanding the effect of the SFP on SCL. In addition, careful interpretation of the results is required and the study should be replicated in future.

Another limitation is that this study only examined associations between behavioural reactions and physiological reactions to the SFP. In addition, due to the excessive amount of correlations the chance on type I errors is high: some relations that are suggested to be significant may not exist at all. Therefore the results should be interpreted carefully. In the future behavioural reactions and physiological reactions should be examined simultaneously. This is also the case for the autonomic nervous system. In the current study the parasympathetic nervous system and the sympathetic nervous system were viewed as two separate systems. In the future, there should be studies which investigate the operation of the two systems simultaneously to examine the co-operation of the two systems.

In addition, in the future the influence of risk status on heart rate and SCL should be examined further. Especially because the SCL levels in this study were suspicious. Also, the effect of the difference between the two risk groups should be investigated in a follow-up to be able to assess the effect of these vulnerabilities in the functioning of the autonomic nervous system on behaviour problems. Furthermore, there should be attention paid to the development of intervention to prevent that these infants develop psychopathology.

Conclusion and Implications

The current study indicated that the SFP was able to elicit sympathetic and parasympathetic activity in 6-months old infants. An increase in sympathetic activity was found during the still-face by changes in heart rate and SCL, but not in PEP. In addition a decrease in parasympathetic activity during the still-face was found by changes in heart rate and RSA. In the transition to reunion, a variety of changes was found. There was no difference in PEP, nor in RSA. Heart rate increased for infants with a higher risk status, while it decreased in infants with a low risk status. In addition, the SCL increased in infants with a higher risk status, while it did not in infants with a low risk status.

According to the current study it can be suggested that 'adverse' mother characteristics, already have their influence on the autonomic nervous system in infants of 6 months of age. This is important because early dysfunctioning of the stress system may have an influence on later outcomes. Difficulties in emotion regulation at a very early age increase the risk for internalizing and externalizing problems in the future (Keenan, 2000) .

Earlier studies have already shown that the SFP is able to evoke an emotional response in infants and that it is a suitable procedure to assess emotion regulation at an early age. This study has added to this literature by giving evidence of a physiological response to the SFP. In addition, if the patterns are explored furthermore, it may be possible to differentiate children of different risk status trough the infant's reaction on the SFP.

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