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Physiological Effects of Separation and Reunion in Relation to Attachment and Temperament in Young Children

ABSTRACT: *This study examined physiological effects of separation and reunion in a sample 3- to 6-year-old children. Using continuous ambulatory recording, changes in heart rate (HR), respiratory sinus arrhythmia (RSA), and pre-ejection period (PEP) were compared across the episodes of a separation–reunion procedure based on the strange situation. RSA decreased significantly over the course of the procedure as well as on separation from the parent and not the stranger, supporting that separation from the attachment figure elicited vagal withdrawal in young children. The absence of significant PEP effects suggest that the separation–reunion procedure, and more specifically separation from the parent, was not threatening enough to activate the sympathetic nervous system, even if children were insecure attached and inhibited with regard to strangers. Some of the variability in HR increases to reunion was explained by younger age. The findings highlight the role of the ANS as a regulatory process in the parent–child relationship. © 2007 Wiley Periodicals, Inc.* Dev Psychobiol 49: 119–128, 2007.

Keywords: *attachment; heart rate; respiratory sinus arrhythmia; pre-ejection period; autonomic nervous system; children*

INTRODUCTION

From an attachment theoretical perspective, separation from a primary caregiver is a natural cue to danger, leading to activation of the attachment system. Activation of the attachment system is followed by changes in behavior, which are supposed to terminate when contact with the attachment figure is re-established (Bowlby, 1969/1997). Behavioral reactions to separations and reunions are expected from all children, although they may be less obvious in some children, for example avoidant attached children, or awkward in others, such as disorganized attached children (Ainsworth, Blehar, Waters, & Wall, 1978; Main & Solomon, 1990). Less is known about concomitant biological reactions. Hofer (2006) has suggested that in fact, there may be multiple regulatory processes operative within the parent–child interaction, that may become temporarily dysregulated as a result of separation. The autonomic nervous system may be involved in the generation of affective and behavioral reactions to separation, and the regulation of emotion on reunion (Fox & Card, 1999). Although the attachment system is assumed to be active in all children when they are separated from their attachment figures, and during the reunion, it is not yet known to what extent the autonomic nervous system is also involved in producing a response, and which parts of the autonomic nervous system, the parasympathetic or the sympathetic, are most involved. Also little is known about the associations between individual differences in these physiological responses and quality of attachment and temperament of children.

First empirical evidence for the link between attachment and physiological processes, comes from animal studies, although primarily focusing on the role of neuroendocrine systems (Carter, 1998). Hennessy

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(1997) reviewed experiments examining the activity of the HPA system as a consequence of brief involuntary separation. HPA response to separation occurred in a number of species, but only when there were also behavioral signs of emotional attachment. In addition to reactivity on separation, physiological responses on reunion may provide insight in the ability of the attachment figure to mitigate stress responses to threatening or fearful situations (Mason & Mendoza, 1998). Coe, Mendoza, Smotherman, and Levine (1978), for example, observed in a study with squirrel monkeys that elevated levels of plasma cortisol after brief separation from mother were only reduced by the reunion with its mother and not by the presence of familiar female animals.

Human studies on HPA reactivity have revealed increasing cortisol levels in infants when they were separated from the mother and left with an unfamiliar babysitter, compared to being alone with the mother (Gunnar, Larson, Hertsgaard, Harris, & Broderson, 1992). In addition, higher cortisol levels were found for insecurely attached infants (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Spangler & Grossman, 1993; Spangler & Schieche, 1998) and for infants with a combination of inhibited approach (e.g., to novelty of social situations) and insecure attachment (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996).

The first empirical study on the link between cardiac reactivity and attachment in humans was done by Sroufe and Waters (1977), who found in a series of case-studies of avoidant attached children that heart rate (HR) accelerated on separation, remaining elevated until reunion with the parent. Spangler and Grossman (1993) found an average HR increase on the second separation, when the child was left alone, with highest HR scores for the disorganized infants. In a study on autistic behavior and attachment, Willemsen-Swinkels, Bakermans-Kranenburg, Buitelaar, Van IJzendoorn and Van Engeland (2000) found HR increases on leavetaking and HR decreases on reunion with the caregiver for disorganized attached children but not for children with a Pervasive Developmental Disorder (PDD). However, a recent study with adolescent mothers and their infants (Zelenko et al., 2005) found no major differences between the attachment groups in infants HR changes during separations and reunions with mother. All these studies focused on infants. Only Stevenson-Hinde and Marshall (1999) studied cardiac reactivity to a modified strange situation of children of preschool age. The physiological measures were the interval between the heartbeats (heart period, HP) and the respiratory sinus arrhythmia (RSA), which reflects parasympathetic activity. The concept of inhibition used in this study referred to a child's initial withdrawal to unfamiliar or challenging events. As expected, HP increased significantly on reunion, except for the high inhibited and insecurely attached children. In addition, RSA also increased on reunion, except for the high-inhibited children. These studies suggest that separations from attachment figures and exposure to strangers may lead to increased cardiac reactivity of young children, whereas reunion with the attachment figure may lead to decreased cardiac reactivity. Furthermore, both relationship characteristics (quality of attachment) as well as individual characteristics (inhibition) appear relevant to understand individual differences in physiological reactivity.

The current study was aimed to extend these earlier studies by examining physiological effects of separation and reunion in older children and by using a wider range of physiological indices. HR reactivity was measured, as well as parasympathetic activity or withdrawal as indexed by respiratory sinus arrhythmia (RSA) and sympathetic activity or withdrawal as indexed by pre-ejection period (PEP). RSA has been found to be a noninvasive indicator of vagal control of the heart, provided that significant variations in respiratory activity are controlled for (Cacioppo, Uchino, & Berntson, 1994). PEP, as derived from systolic time intervals, is a non-invasive marker of sympathetic control of the heart, at least when used to examine within-subjects changes (Cacioppo et al., 1994; De Geus & Van Doornen, 1996).

From a theoretical perspective, it has been suggested that information about the different parts of the autonomic nervous system that are involved in arousal could contribute to the understanding of adaptive and maladaptive behavior. An important theoretical proposal was made by Porges (2004), who described the functioning of the social engagement system in relation to the vagal system. The vagal system originates in the nucleus ambiguus and functions to maintain homeostasis. When there are challenges to homeostasis, the vagal system allows individuals to show social engagement behaviors that promote social communication, by regulating vagal input to the heart and support the changes in HR required for these behaviors (the vagal "brake"; Porges, Doussard-Roosevelt, & Greenspan, 1996). When the vagal "brake" is not applied, other more primitive neural systems, such as the sympathetic nervous system are recruited. The sympathetic nervous system is a mobilization system that fosters fight and flight behaviors (Porges, 1997). An important contextual component that determines whether a person will show social engagement or defensive behavior is the perception of safety. If the context is perceived as safe, then the primitive neural systems are inhibited to allow the expression of social engagement (Porges, 2004). Because separation–reunion procedures are supposed to induce only mild distress (Ainsworth et al., 1978), being separated from the attachment figure and left alone or with a stranger should elicit activity from the vagal system whereas the sympathetic nervous system might be less active. However, individual differences are expected in the development of these regulatory systems as a result of the interaction between children and their environment. Children may differ in their mode of autonomic response to challenges (Cacioppo et al., 1994).

This study focused on physiological responses of children during a separation–reunion procedure, which was designed to engender accumulating stress. It was predicted that HR would increase and PEP and RSA would decrease from the start until the end of the procedure. There were four main questions: (1) Would separation and reunion with the attachment figure be reflected in changes in HR, RSA, and PEP? HR was expected to increase on separation. Furthermore, we expected changes in RSA and PEP which included parasympathetic withdrawal as reflected by RSA decreases, sympathetic activation as indexed by a shortened PEP or a combination of both (Cacioppo et al., 1994). In contrast, reunion with the attachment figure may elicit HR decreases and parasympathetic activity, as indexed by RSA increases, sympathetic withdrawal as reflected by higher PEP values or a combination of both. In addition, if HR effects were found while PEP and RSA did not change, post-hoc analyses should be done to examine whether the variance in HR changes on separation and reunion can be accounted for by PEP and RSA. (2) Are the physiological reactions specific for engagement with attachment figures and not for engagement with an unfamiliar adult? The specificity of the physiological effects were tested by comparing reactions to parent and stranger. We expected that physiological responses to separation and reunion with the parent were stronger than responses to separation and reunion with a stranger. (3) To what extent would individual differences in physiological responses to separation and reunion be explainable from the quality of the attachment relationship and from temperamental characteristics, such as behavioral inhibition? (4) To which extent do children show autonomic responses to separation and reunion consistent with a model of regulation?

METHOD

Participants

The sample consisted of 50 parents (48 mothers) and their biological children (28 girls) between the ages of 3 to 6 years ($M = 54.7$ months, $SD = 9.25$). Of all the 50 families, 28 were registered by foster care agencies indicating that the families had also foster children in their home or were intended to provide foster care in the near future. These families had been recruited through foster care agencies in Amsterdam, Rotterdam, and Utrecht (the Netherlands) as part of a larger study comparing attachment of children to foster parents and biological parents. The remaining 22 families had been recruited by schools and day-care centers in Amsterdam and the surrounding area. Results were checked for differences between these two groups of parents but no significant differences were found. Informed consent was obtained from all the families before participation.

Procedure

All parent–child dyads were observed twice within 2 weeks, once at home and once, 2 weeks after the home-visit, at the university for a separation–reunion procedure. Parent and child were visited in their homes by a trained observer for 2 hr. During the last 30 min, parent and child were asked to play with the toys that the observer had brought along. After the home-visit, the trained observer sorted the 90 items of the Attachment q-set (Waters, 1995) on the basis of the observations during the visit. After 2 weeks, the parent and child visited the university laboratory for the separation–reunion procedure. Because the original strange situation procedure (Ainsworth & Wittig, 1969) does not yield parallel separation and reunion episodes for parent and stranger, the procedure was extended based on guidelines for coding preschool attachment (Cassidy & Marvin, 1992). This modified procedure takes 24 min and involves 10 episodes, which were represented in Figure 1. Physiological measures were conducted during the whole separation–reunion procedure. Movements were reduced by seating the children behind a table with toys. Each child wore six electrodes on the skin which were connected to a small lightweight device (VU-AMS). The children were given a jacket to wear, that had a pocket carrying the device.

Measures

Quality of Attachment. Quality of attachment was assessed with the Attachment q-set (AQS), Version 3 (Waters, 1995). The AQS consists of 90 descriptions of child attachment behavior supplemented with so-called filler-items which make the focus on security less obvious. After the home-observation, the 90 attachment relevant items were sorted by a trained observer in nine piles, according to a predefined rectangular distribution. A security score is obtained by correlating the child's q-sort with an expert criterion sort for a prototypically secure child (Waters & Deane, 1985). Security scores may thus range from -1.00 for the most insecure child to $+1.00$ for the most secure child. The inter-rater reliabilities for two observers with the scores of two experts exceeded .90 for both observers. Later reliability check on independent sorts of 18 children revealed an intraclass correlation of .70 between the two observers.

The validity of the AQS was tested in a meta-analysis on 139 studies with 13835 children (Van IJzendoorn Vereijken Bakermans-Kranenburg & Riksen-Walraven 2004). Results revealed convergent validity with the strange situation ($r = .31$) and discriminant validity with temperament ($r = .16$).

Inhibition to Strangers

An observational inhibition scale was constructed on the basis of the Attachment q-set (AQS) (Waters, 1995). Although the AQS

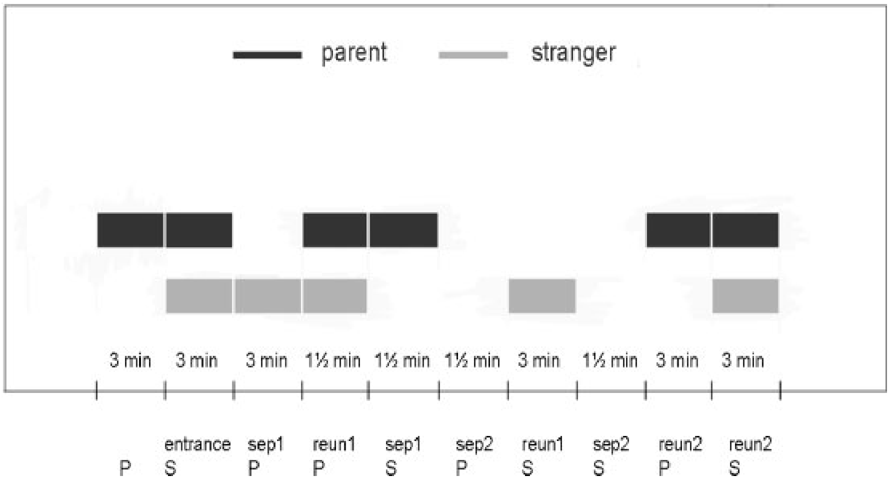

FIGURE 1 Episodes of the separation-reunion procedure including separation (sep) and reunion (reun) with parent (P) and stranger (S).

was developed primarily to assess attachment security, q-set methodology allows for the simultaneous assessments of multiple characteristics at once, which can be analyzed at the level of single items or on the level of combinations of items (Block, 1961). In developing the AQS, Waters and Deane (1985) had included a set of items tapping temperamental characteristics. The following items of the AQS were selected to tap inhibition to strangers: *item 7* Child laughs and smiles easily with a lot of different people (low: Mother can get him to smile or laugh more easily than others), *item 12* Child quickly gets used to people or things that initially made him shy or frightened him (middle: if never shy or afraid, low: child is slow to get used to people or things), *item 66* Child easily grows fond of adults who visit his home and are friendly to him (low: does not grow fond of new people very easily).

The value for coefficient alpha for these scale was .85, indicating satisfactory reliability. Further, the scale was orthogonal to attachment security ($r = .09$). To examine convergent validity, a correlation coefficient was computed between this scale and the scale *shyness* of the Children's Behavior Questionnaire (CBQ) (Dutch translation: Van den Bergh & Ackx, 2003; Rothbart, Ahadi, Hershey, & Fisher, 2001), a measure of child temperament for children between the ages of 3 and 7 years. *Shyness* was defined as slow or inhibited approach in situations involving novelty or uncertainty (Rothbart & Ahadi, 1994). Of all the parents, 41 completed the questionnaire. The correlation between the AQS shyness scale and the CBQ shyness scale was .37 ($p < .05$).

Physiological Measures

The Vrije Universiteit-Ambulatory Monitoring System 46 (VU-AMS) was used to measure HR, RSA and PEP during the separation-reunion procedure. Six disposable (neonatal/pediatric) Ag/AgCI electrodes were used to record continuously electrocardiogram (ECG), basal thorax impedance (Z_0), changes in impedance (dZ) and the first derivative of pulsatile changes in transthoracic impedance (dZ/dt). To yield the impedance cardiogram (ICG), dZ/dt is sampled at 250 Hz (De Geus & Van Doornen, 1996). The VU-AMS software programs (http://www.psy.vu.nl/vu-ams) were used to extract the physiological indices. First, a label data file was created to divide the entire measurement period into separate periods. The video recordings were used to define start and stop times of the episodes of the separation–reunion procedure as well as other information about physical activity and mood state.

The software program (AMSIMP) for impedance cardiogram (ICG) derived and displayed an average ICG waveform of 128 samples (512 milliseconds). The following three points were automatically scored and marked on the average dZ/dt waveform, (1) B-point or upstroke, (2) $dZ/dt_{(\mathrm{min})}$, and (3) X-point or insicura. From these points, the pre ejection period (PEP) was obtained, which is defined as the time between the onset of ventricular depolarization (Q wave onset) and the onset of left ventricular ejection of blood in the aorta (B-point) (De Geus, Willemsen, Klaver, & Van Doornen, 1995). Because of the limited reliability of B-point detection, due to ambiguity in the location of the B-point, each dZ/dt waveform was checked and corrected or deleted when automated scoring revealed B-points that were morphologically inconsistent (Riese et al., 2003). Fewer than 5% of the waveforms were discarded. The procedure of interactive visual inspection of the waveforms was done by trained research staff. Inter-rater reliability was determined on a combined data set of 15 subjects with 2377 ICG signals. Pearson correlation coefficients between the three raters ranged from .88 to .94 (intraclass correlation coefficient was .90). Later reliability checks on recordings of nine subjects (578 ICG signals), that resemble less of the typical morphology of a ICG signal, showed an intraclass correlation coefficient of .89.

The AMSRES software program for continuous measurement of ECG R-wave to R-wave intervals and thoracic impedance) was used to view and correct the respiration signal. The respiration signal was obtained from filtered (0.1–0.4 Hz) thoracic impedance signal. The begin and end of inspiration and expiration was detected by an automatic scoring algorithm built into the AMSRES program. RSA was derived by the peak-through method

(Grossman, Van Beek, & Wientjes, 1990) which combined the respiratory time series and the inter beat intervals (IBI) to calculate the shortest inter beat interval during heart rate acceleration in the inspiration phase, and the longest inter beat interval during deceleration in the expiration phase (De Geus et al., 1995). RSA was defined as the difference between the longest and the shortest IBI. The RSA is set on zero or a negative value was obtained for a particular breath cycle, when the longest or shortest IBI was missing for that breath cycle. Although the AMSRES program automatically scored the respiration signal, interactive scoring of the respiration signal and the IBI was also allowed. Inappropriate parts of the signals were rejected manually.

RESULTS

Effects of Separation and Reunion on HR, lnRSA, and PEP

To determine the effects of the separation–reunion procedure on HR and ANS functioning, repeated measures ANOVAs were performed for each physiological variable (HR, RSA, and PEP) to identify significant increases and decreases across the episodes. Because RSA was skewed at all the episodes of the procedure, its natural logarithm was used in the analyses. The results are presented in Figure 2. Significant main effects for episodes on HR, Wilk's $\Lambda = .50$, $F(5,45) = 8.98$, $p < .001$, $\eta^2 = .50$ and lnRSA, Wilk's $\Lambda = .70$, $F(5,45) = 3.84$, $p < .01$, $\eta^2 = .30$, indicated significant changes across the episodes, except for PEP. Planned contrasts were used to examine whether HR, lnRSA, and PEP changed across the entire separation-reunion procedure. As expected, lnRSA significantly decreased from the begin until the end, $F(1,49) = 14.07$, $p < .001$, indicating an increase in parasympathetic withdrawal during the procedure. Contrary to expectations, there was no difference for HR and PEP change from the start till the end of the procedure.

Planned contrasts were also used to examine effects of separation and reunion. Only the separation- and reunion-episodes with the parent were included because there were no directional hypotheses about physiological reactions to separation or reunion with a stranger. Analyses showed a significant increase in HR during the first separation from the parent, $F(1,49) = 19.77$, $p < .001$ and a significant decrease of lnRSA, $F(1,49) = 4.18$, $p < .05$. Additionally, lnRSA decreases on the second separation from the parent were marginally significant, $F(1,49) = 3.33$, $p = .07$. There was no significant change in HR on the second separation from the parent. Planned contrasts on the first en second reunion revealed that HR increased significantly on the second reunion with the parent, $F(1,49) = 7.67$, $p < .01$. Contrary to expectations, there were no significant changes in lnRSA on the reunions.

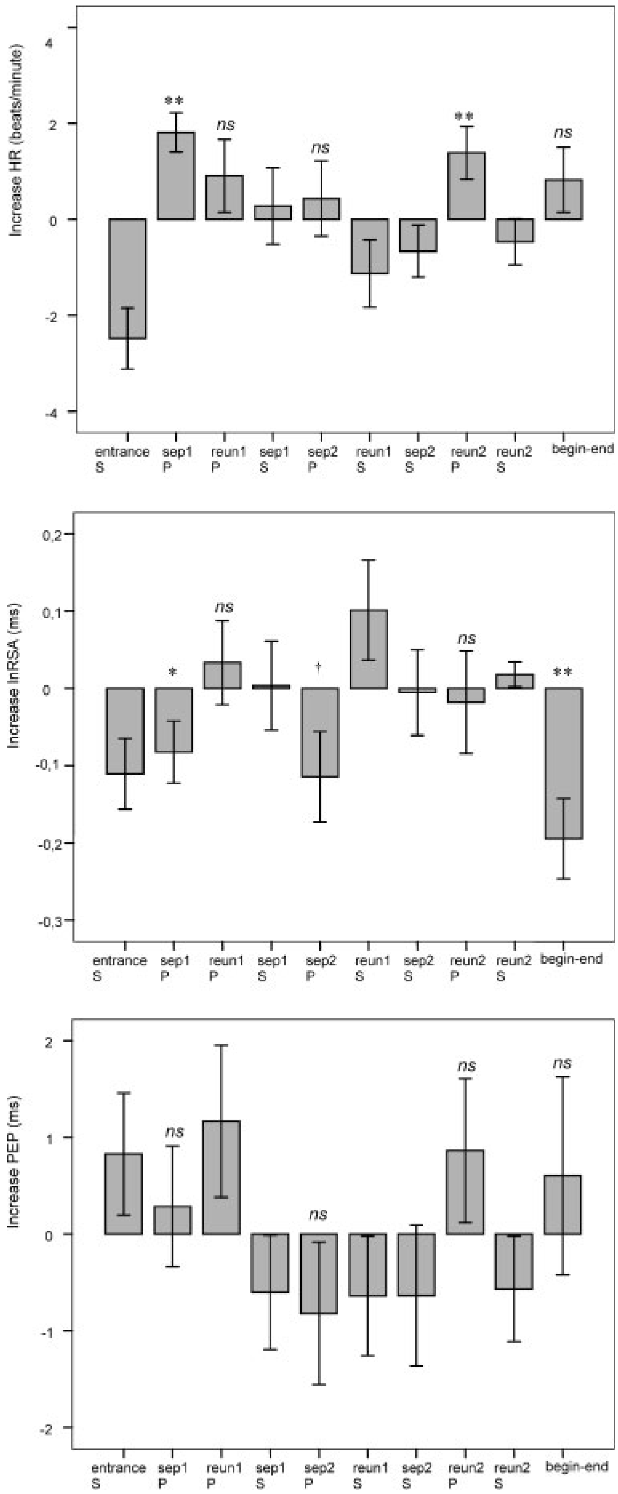

FIGURE 2 Mean HR, lnRSA, and PEP increases across the episodes of the separation-reunion procedure. $^{\dagger}p < .10$, $^{*}p < .05$, and $^{**}p < .01$ represents significantly increase or decrease on separation (sep) and reunion (reun) with parent (P) or stranger (S). Error bars depict SEM.

Finally, PEP changes across the different separation and reunion episodes were not significant.

To examine the amount of variance in HR that the parasympathetic nervous system and the sympathetic nervous system accounted for, regression analyses were performed with lnRSA and PEP as predictors. The linear combination of mean lnRSA and PEP increases were not significantly related to mean HR increases on separation and on the first reunion. However, examinations of individual predictors indicated that PEP was significantly related to HR on the first reunion, $b = -.27$, $t = -1.98$, $p = .05$. The prediction of mean HR increases on the second reunion from the linear combination of mean lnRSA and PEP increases was significant, $R = .48$, $F(2,47) = 6.91$, $p < .01$ and accounted for 23% of the variance in HR increases. Additionally, analyses of individual predictors revealed that both lnRSA ($b = -3.23$, $t = -3.02$, $p < .01$) and PEP ($b = -.23$, $t = -2.44$, $p < .02$) were significantly associated with HR on the second reunion.

Specificity of HR, lnRSA, and PEP Reaction to Separation and Reunion With Parent and Stranger

First, change scores were computed for each child, by subtracting the two succeeding episodes on separation and reunion. Positive values for change therefore reflect increase in the indicators of autonomic regulation. The mean increase scores on separation and reunion from both the parent and the stranger are presented in Table 1. Repeated measures ANOVA were conducted for each physiological mean increase score, to compare responses to stranger and parent. HR increases on the first separation from the parent were significantly higher than HR increases on the first separation from the stranger, $F(1,49) = 4.10$, $p < .05$. In contrast, there were no significant HR differences on the second separation from the parent and the stranger. The comparisons concerning mean lnRSA and PEP increases on separation were not significant. With respect to reunion, analyses revealed a significant HR effect, $F(1,49) = 6.56$, $p < .01$, that is, HR increased more on the second reunion with the parent than with the stranger. The HR effect on the first reunion was in the same direction but approached significance, $F(1,49) = 3.50$, $p = .07$. Similarly, the mean PEP increase on the first reunion approached significance, $F(1,49) = 3.34$, $p = .07$. Thus, HR and to a lesser degree PEP, increased more on reunion with the parent than with the stranger. Results revealed no significant lnRSA differences on reunion with parent and stranger.

Individual Differences in HR, lnRSA, and PEP in Relation to Attachment and Temperament

A multiple regression analysis was conducted to predict mean increases of the different physiological measures on separation and reunion with the parent. The predictors

Table 1. Mean Increase and Standard Deviations (SD) for HR, lnRSA, and PEP on Separation and Reunion with Parent and Stranger ($N = 50$)

	Parent	Stranger			
Measure[a]	*M* (SD)	*M* (SD)	*df*	*F*	*p*
First separation					
HR	1.81 (2.88)	.28 (5.62)	1	4.10*	.048
lnRSA	−.08 (.29)	.00 (.41)	1	1.71	.20
PEP	.28 (4.42)	−.60 (4.17)	1	1.20	.28
Second separation					
HR	.43 (5.52)	−.66 (3.82)	1	1.50	.23
lnRSA	−.11 (.41)	−.01 (.39)	1	2.21	.14
PEP	−.82 (5.23)	−.64 (5.16)	1	.04	.85
First reunion					
HR	.91 (5.37)	−1.13 (4.98)	1	3.50†	.07
lnRSA	.03 (.39)	.10 (.46)	1	.60	.44
PEP	1.17 (5.57)	−.64 (4.39)	1	3.34†	.07
Second reunion					
HR	1.38 (3.90)	−.47 (3.40)	1	6.56**	.01
lnRSA	−.02 (.47)	.02 (.11)	1	.29	.60
PEP	.86 (5.26)	−.57 (3.85)	1	2.32	.14

†$p < .10$, *$p < .05$, and **$p < .01$.

[a]HR, heart rate (in beats/min); RSA, respiratory sinus arrhythmia (in logs); PEP, pre-ejection period (in ms).

were child's age, gender, and temperament in terms of behavioral inhibition and security of attachment in the first step. Step 2 added the interaction between behavioral inhibition and security of attachment. For the mean HR increases on the first separation as a dependent variable, only the regression equation with the interaction variable accounted for a significant proportion of the variance, R^2 change $= .08$, $F(1,44) = 4.18$, $p < .05$. The interaction between behavioral inhibition and security of attachment indicated that low inhibited, high secure children on the one hand and high inhibited, low secure children on the other hand tended to showed greater HR increases on separation than low inhibited, low secure and high inhibited, high secure children. However, examining Mahalanobis distances, one multivariate outlier was identified ($p < .001$; Tabachnick & Fidell, 2001). With this outlier deleted, the interaction effect did not remain significant ($p = .46$). The prediction of mean HR increases on the second separation was not significant.

Analyses with the mean HR increases on the first reunion as a dependent variable indicated that the first regression equation was not significant. However, the regression equation with the interaction variable accounted for a significant proportion of the variance, R^2 change $= .10$, $F(1,44) = 5.10$, $p < .05$. This interaction effect indicated that only the high inhibited, low secure children decreased in HR on reunion. But one case was identified through Mahalanobis distance as a multivariate outlier with $p < .001$. Regression analysis without this case revealed no significant interaction effect ($p = .27$) With regard to mean HR increases on the second reunion, the regression equation was significant, R^2 change $= .22$, $F(4,45) = 3.08$, $p < .03$. Examinations of individual predictors indicated that the main effect for age was significant ($t = -2.69$, $p < .02$) whereas the effect of inhibition was marginally significant ($t = -1.83$, $p = .07$), indicating that younger and low inhibited children tended to show higher HR increases on the second reunion. The predictors gender and security were not significant. Further, regression analyses with mean lnRSA and PEP increases as dependent variables revealed no significant results.

Patterns on the Level of Individual Children

One-tailed, z approximation tests were conducted to assess whether the percentages of children that responded on at least one physiological indicator in the expected direction exceeded chance level (75%). Expected physiological responses were lnRSA and PEP decreases on separation and lnRSA and PEP increases on reunion. Table 2 presents the percentages of children that responded with none, both of one of the physiological indicators in the expected direction. Analyses were done with the percentages of children that responded with one or both physiological indicators in the expected direction. The observed percentage children that responded with lnRSA or PEP decreases on the first separation, did not significantly differ from 75% whereas the observed percentage (86%) children that responded with lnRSA or PEP decreases on the second separation exceeded the 75% chance level ($p < .05$, one-tailed). On the first reunion, the observed percentage (86%) children that responded with lnRSA and/or PEP increases also exceeded chance level ($p < .05$, one-tailed), which was not the case on the second reunion.

Table 2. Percentage Children with Expected lnRSA and PEP Responses on Separation and Reunion with Parent

	lnRSA and PEP Response in Expected Direction			
	None	One	Both	One & Both
Separation 1	24	52	24	76
Separation 2	14	30	56	86*
Reunion 1	14	58	28	86*
Reunion 2	18	50	32	82

*$p < .05$, one-tailed, represents significant differences from chance level (75%).

DISCUSSION

Children in a separation–reunion procedure based on the strange situation showed significant autonomic reactivity, indicated by lower RSA. Within the procedure the coming and going of the attachment figure did not universally lead to measurable physiological responses but most children showed expectable autonomic responses to separation and reunion. The expected effects of separation on physiological arousal were significant for HR and RSA, when children were left with the stranger. Effects of reunion with the attachment figure, as well as differences between reactions to separation and reunion from parent and stranger were significant for HR, but not in the expected direction for the reunion episodes.

The significant increase of HR and decrease of RSA on the first separation from the parent indicates that being left by the attachment figure with stranger challenges the vagal system. Effects of being left alone were less evident for RSA and, in contrast with a previous study (Spangler & Grossman, 1993) absent for HR. However, Zelenko et al. (2005) found no significant effects on separation at all, although they only tested on HR. Differences between HR responses to parent and stranger were found on the first separation, showing that HR increased more on separation from the parent than on separation from the stranger. The absence of PEP reactivity on separation indicates that the sympathetic system was not activated.

There was no significant increase in RSA on reunion with the parent, which would be expected on the basis of the study of Stevenson-Hinde and Marshall (1999). Unexpectedly, HR increased during the second reunion with the parent and this was also shown in comparisons between parent and stranger, indicating that HR increased more on reunion with the parent than on reunion with the stranger. Other studies reported no significant HR effects (Spangler & Grossman, 1993; Zelenko et al., 2005), but one study reported HP increase (HP and HR are inversely related) on reunion (Stevenson-Hinde & Marshall, 1999). The most important difference between our study and the study of Stevenson-Hinde and Marshall is the length of separation. The longer separation (25 min in contrast to 6 min at maximum in our study) in the study of Stevenson-Hinde and Marshall may have resulted in stronger activation of the attachment system which in turn would have led to more relief on reunion as measured by HR responses. On the other hand, the increase in HR on reunion may be the result of other changes from separation to reunion, for example speech, muscular tension and locomotion, although the latter is less likely because we reduced movement by seating the children behind a desk. It should be noticed that HR is more sensitive to such potential confounds than RSA and PEP, and therefore the latter are increasingly preferred in studies of autonomic response (Cacioppo et al., 1994).

The variability in HR effects on reunion could partly be explained by age, indicating higher HR increases for younger children. Additionally, low inhibited children also tended to show higher HR increases on reunion. Stevenson-Hinde and Marshall (1999) demonstrated that only low inhibited, secure children showed HP increases (HP increases reflects HR decreases) on reunion. The combination of security of attachment and temperamental inhibition was also significantly related to HR on separation and reunion in the current study, but the dependence of this effect on outlying cases casts doubts over the replicability of this finding. Individual differences in RSA and PEP responses could not be explained by age, temperament, or security of attachment.

Overall, what is striking in the findings of the current study is the lack of PEP responses which suggests that the separation–reunion procedure, and more specifically separation from the attachment figure, was not threatening enough to activate the sympathetic nervous system, even if children were insecure attached and inhibited with regard to strangers. In terms of Porges model of a social engagement system (Porges, 2004), these separations might have induced only nonthreatening challenges to homeostasis (e.g., focused attention or social interactions), which the vagal system should be able down regulate (by removing the vagal "brake"), resulting in rapid increases of cardiac output (RSA decreases and HR increases) without activation of the sympathetic nervous system. It is possible that for some children such separation are more frightening, for example, children with a history of neglect or frequent changes of primary caregivers. Studies of at risk populations might be done to assess whether some children might in fact show responses in the sympathetic nervous system to being left alone by a (foster) parent or with a stranger. Although separation elicited no sympathetic activity, differences between PEP reactivity on the first reunion with the attachment figure and the stranger revealed a marginally significant effect, indicating higher PEP increases (sympathetic withdrawal) on reunion with the attachment figure. Other differences between the attachment figure and the stranger were only significant for HR, which indicates that there were no differences between autonomic regulation on separation and reunion with attachment figures as compared to strangers, at least as regards regulation of the parasympathetic nervous system. This may suggest that the social engagement system is not exclusively for parent–child relationship, but may also be active during other social interactions.

In conclusion, our findings showed that activation of the attachment system in young children led to autonomic nervous system responses. In general, effects of separation could be mainly ascribed to parasympathetic withdrawal whereas the physiological responses on reunion were more difficult to ascribe to a specific part of the autonomic nervous system. Nevertheless, we found that changes in physiological arousal occur in a nonrandom patterns, under the influence of experimentally induced separations and reunions with the parent and a stranger. These results suggest that there is a normative pattern of physiological responses to separation and reunion with the parent, and that biological reactions in the ANS accompany activation of the attachment behavioural system. Further research is needed into the interplay between attachment behavior and ANS activity. Some children's physiological responses were not in the expected direction. Research with physiological measures on dyads with not yet established or extremely insecure or disordered attachment relationships (e.g., institutionalized settings, foster care, adoption) may reveal more information about the psychological meaning of these physiological responses.

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