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Maternal separation and contact to a stranger more than reunion affect the autonomic nervous system in the mother-child dyad

ANS measurements during Strange Situation Procedure in mother-child dyad

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ABSTRACT

Background: The autonomic nervous system (ANS) processes underlying attachment-related mother-child interactions are not well understood. We aimed to describe and compare the responsivity of the ANS for mothers and their infants during the different phases of the Strange Situation Procedure (SSP).

Methods: Continuous measurements of the sympathetic (SNS) and parasympathetic (PNS) branches of the ANS were obtained simultaneously in 91 mothers and their infants (range 10–15 months). Heart rate (HR), respiratory sinus arrhythmia (RSA), pre-ejection period (PEP) and left ventricular ejection time (LVET), were calculated for the baseline period (e1) and seven subsequent episodes (e2–e8) of the SSP.

Results: The largest difference between the mother and infant was during e7, when the stranger went into the room where the infant was while the mother waited outside the room. Mothers showed reduced SNS-activity or stress reduction while the child showed PNS withdrawal or increased stress response. Additionally, LVET was found to be a marker for SNS changes in the one-year-old infant during SSP.

Conclusion: Mothers and infants showed different stress-related ANS responses during e7. Since this study showed that simultaneous measurement of ANS responses in mother-child dyads during the SSP is feasible, future studies can assess both mother and child stress responses in different contexts. The measure of LVET may be a valid SNS-reactivity measure in the one-year-olds. Since the separation episode e7 led to the strongest ANS responses, future studies might assess stress responses in more normative circumstances, such as child care programs.

1. Introduction

Reciprocal mother-child interactions are influenced by cognitive, emotional and physiological regulatory processes that can be disturbed under conditions of maternal separation (Hofer, 2006). This leads to attachment system activation with specific emotional and behavioral, stress-related reactions in both, mothers and their infants depending on the attachment style. These reactions may be transmitted (epi-) genetically and enable the transmission of early life experiences across generations (Bowers and Yehuda, 2016).

In the 1950s John Bowlby defined the attachment system as a neurobiologically controlled inner model of how relationship works (Chambers, 2017). Individual attachment behavior depends on infant experience in contact with the caregiver. Aside from that, it is influenced by the fact, if, and in what degree a situation is perceived as secure or threatening from the maternal and the infant point of view (Bowlby, 1980). The notion of individual differences in attachment was investigated by Mary D. S. Ainsworth during the Strange Situation

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Procedure (SSP). This procedure induces attachment related stress by separation and reunion of a mother-child-dyad (Ainsworth, 1978; Crittenden, 2017).

On the physiological level, the above mentioned perception of safety or threat influences the autonomic nervous system (ANS) with its sympathetic and parasympathetic branch (Oosterman and Schuengel, 2007b; Porges, 2003).

This is important as the inner model of attachment of an infant continues throughout life as well as the individual variability of the ANS, and therefore adaptability to stress. Both affect, later in life, physical and mental health (Chambers, 2017).

A variety of brain imaging studies of mother-child interactions identified hypothalamic-midbrain-limbic-paralimbic-cortical circuits as the neurobiological basis of attachment (Swain et al., 2007). On the other hand, little is known about how attachment related stress influences the responses of maternal and infant ANS separately and how they influence each other.

The aim of this study was to provide new evidence on the effect of attachment related stress during SSP on the different branches of the ANS to illuminate the divergent autonomic responses within the mother-child dyad.

1.1. The ANS in adult and infant

The ANS is mainly involved in emotional and behavioral reactions initiated by an attachment stressor (Cassidy and Shaver, 1999; Hill-Soderlund et al., 2008) and it is controlled by the central autonomic network (CAN) (Critchley et al., 2011). The CAN is directly linked to the regions of the brain affected by attachment (Feldman and Ellenberger, 1988). Alterations in the ANS are detectable far in advance of the awareness of specific emotions and therefore it is possible to measure early, unconscious and body-related arousal using the ANS (Garfinkel et al., 2016).

ANS responses under resting and challenging conditions indicate an individual's ability to maintain internal homeostasis and enable the body to respond to external impulses perceived as stressors. A model described by Porges explains, that the PNS (vagal reactions) consists of two motor systems: while the dorsal motor nucleus of the vagus provokes immobilization by decreasing heart rate (HR), the nucleus ambiguous sustains homeostasis by restricting the sympathetic branch and enables social contact and communication by the “vagal break”, which is active when somebody begins to feel safe (Porges, 2003, 2007; Oosterman et al., 2010). This “Polyvagal Theory” shows a relationship between ANS function and social behavior and is one of the ongoing hypotheses concerning the regulation of autonomic state. The perception of a situation as “safe” or “threatening” determines whether a person will show fear behavior via the SNS or social engagement via the PNS (Porges, 2003, 2007; Berntson et al., 2007).

In adults, ANS measures have been successfully investigated in relation to behavioral problems and emotion regulation (Lewis et al., 2008).

In contrast to adults, infants differ in their autonomic response to challenges, depending on age, environment, emotion regulation, and attachment quality (Alkon et al., 2011; Smith et al., 2016; Hill-Soderlund et al., 2008; Michels et al., 2013). While resting parasympathetic tone was shown to stabilize at the age of around 12 months, other studies found weak to moderate PNS stability in resting and challenging situations up to the age of 60 months (Alkon et al., 2011; Calkins et al., 2008). Little is known about changes in the SNS in young infants across the ages (Alkon et al., 2011; Alkon et al., 2006; Oosterman and Schuengel, 2007a, 2007b; Oosterman et al., 2010). However, little previous research has measured PNS and SNS simultaneously in infants, for a better understanding of the integrated function of the two ANS branches (Oosterman and Schuengel, 2007a, 2007b; Bush et al., 2016) and no studies simultaneously measured PNS and SNS in both infants and their mothers.

1.2. Measures of the ANS in attachment sensitive settings

Attachment theory (Bowlby, 1982) plays a prominent role in developmental psychology, by identifying the importance of one or more sensitive caregivers for the optimal development of young infants (Van Rosmalen et al., 2015). The Strange Situation Procedure (SSP) (Ainsworth, 1978) has been developed as a research protocol to classify individual infant's attachment-related behavior during the interaction and separation of mothers and their infants. These behavioral and also emotional reactions follow alterations in the PNS and SNS (Garfinkel et al., 2016), that indicate an individual's ability to maintain internal homeostasis and enable the body to respond to external impulses perceived as stressors, e.g. separation from the caregiver (Alkon et al., 2011).

Since the 1970s, heart rate (HR) measures in mothers and infants during separation or reunion have been widely used (Sroufe, 1977; Donovan and Leavitt, 1985; Spangler and Grossmann, 1993; Zelenko et al., 2005). HR is an integrated measure since it is affected by both the PNS and SNS. Recent researchers identified respiratory sinus arrhythmia (RSA), a measure of the parasympathetic nervous system (PNS) and pre-ejection period (PEP), as measure of the sympathetic nervous system (SNS), as more specific ANS measures than HR. RSA reflects the parasympathetic branch of the ANS and is a common index to measure vagal functioning in young infants (Cacioppo et al., 1994; Blair et al., 2013; Alkon et al., 2011; Alkon et al., 2006). PEP measures the sympathetic nervous system's influence on the contractility of the heart (Thayer and Uijtdehaage, 2001). There are few studies of young children's PEP but those that measure PEP find no significant changes in response from challenge conditions to resting conditions (Oosterman et al., 2010; Oosterman and Schuengel, 2007b).

Previous findings though raise the question if PEP is a suitable measure for the SNS in one-year-old infants. Another physiologic measure of the sympathetic branch is the left ventricular ejection time (LVET) (Thayer and Uijtdehaage, 2001). PEP is an inotropic measure of the SNS, while LVET is a chronotropic measure of the SNS. In contrast to PEP, LVET measures in infants during SSP, to our knowledge, have never been investigated.

There are even fewer research studies that measure both infant's PNS and the SNS responsivity during attachment sensitive situations. These ANS measures can be potentially important to help us understand if they function synergistically or orthogonally in relation to a specific stressor, such as maternal-infant attachment (Bush et al., 2016).

We know that the stress regulation abilities of a mother influence the self-regulation capacity of the child (Hofer, 2006; Sroufe, 1997). From the age of six months on, the mother-infant-synchrony, which means a dynamic exchange of impulses within the mother-child-dyad, is very important for infant's self-regulation abilities during stress (Feldman, 2012). The ANS is therefore supposed to play an essential role in this synchrony process (Feldman and Eidelman, 2007; Manini et al., 2013). Previous research, concerning ANS measures during SSP, concentrated on the separation and reunion episodes. In contrast, this study wants to realize ANS measures over the whole SSP: as ANS reaction is a dynamic process, high sympathetic activation e.g. during separation is probably more significant, when linked to the preceding episode.

For the first time, continuous ANS measurements of HR, RSA, PEP and LVET were taken simultaneously in mothers and their one-year-old infants during the entire SSP. We also aim to introduce LVET as a frequency-related SNS representative in infant ANS measures.

The study hypotheses are:

1. The SSP will induce ANS responses in both the mothers and infants across the different episodes.
2. Some, but not all of the SSP episodes will induce PNS withdrawal and/or SNS activation in mothers and their infants relative to the previous episode.

3. There will be a significant difference in the mothers' and infants' ANS responses due to the infants' physiologic development.
4. The SSP will induce significant LVET responses (chronotropic measure of the SNS) in the one-year-old infant relative to the previous episode.

2. Material and methods

2.1. Participants

The “My childhood – Your childhood” study is a cohort study designed to understand the pathways leading to resilience or vulnerability after childhood maltreatment (CM). This study uses a transgenerational approach and includes an interdisciplinary team of scientists from medicine, psychosomatic medicine, psychiatry, psychology and experimental research. This ANS study of SSP is a smaller study, conducted when the infants were 12 months of age, within the larger, cohort study. Between October 2013 and December 2015, 1460 mothers were approached to participate in the study in the maternity unit of the women's hospital of the Ulm University Hospital 1–6 days after parturition. The inclusion criteria were mothers of at least 18 years at the time of their delivery, at least 37 weeks pregnant, infant birth weight of > 2700 g, and mother's fluency in spoken and/or written German. Exclusion criteria included infants that required postnatal intensive care or mothers suffering from psychotic diseases, AIDS, or abusing drugs or alcohol.

Of the 1460 mothers, 533 signed the consent form for participation and completed a screening interview for childhood maltreatment (CM) with the Childhood Trauma Questionnaire (CTQ) (t0) (Bader et al., 2009). Of the 533 mother-child dyads, 240 could be seen for a follow-up 3 months after birth in laboratory as well as in home visit (t1), and 247 participated in a second laboratory visit 12 months after birth (t2).

At t2, complete ANS data were determined all over the SSP in 163 mother-child-dyads. In 91 of these, mothers did not experience childhood maltreatment.

These 91 mother-child dyads (50 male and 41 female one-year-old infants; 12.0 months ± 0.1 months (range 10–15 months)) were included in the following analysis.

If no complete ANS data could be recovered (n = 84), this was mostly (2/3) due to technical failure: participants lost electrodes during SSP, infants denied to place on the mobile unit or removed electrodes, motion artefacts, data transmission failure. About one third of the measures without complete ANS data occurred due to non-compliant mothers, that didn't respect the course of the SSP (didn't leave the room in time, were having a phone call in between, went to the bathroom for a longer time, interrupted for breastfeeding).

Seventy-seven percent of the 91 mothers (32.4 years ± 0.4 years (range 21–43 years)) were married, 23% were unmarried, with 98% lived with a partner. Ninety percent of the mothers had German citizenship. Seventy-three percent of the mothers had a university-entrance diploma or advanced technical college entrance qualification and 64% had completed a polytechnic or a university degree. Twenty-six percent of the mothers had medical risk factors e.g. high blood pressure, allergies or hypo- or hyperthyroidism and 41% had a chronic disease, 14% reported a lifetime diagnosis of a mental health diagnosis (e.g. depression).

2.2. Procedure

Ninety-one mother-child dyads with mothers without CM experience were seen at the Laboratory between 10.00 a.m. and 1.00 p.m. for a separation-reunion experiment, the Strange Situation Procedure (SSP), which was conducted according to the standard protocol developed by Ainsworth (1978).

Before starting the SSP, mother and infant played together for 30 min to approximate a relaxed, resting state, and minimize the activation of the neuroendocrine and catecholaminergic stress axes.

In the first episode (e1), a three-minute baseline episode, the infant was sitting in the mother's lap, listened to a digitally recorded lullaby (Brahms' Lullaby) and faced a laptop screen with visual signs and moving colored bubbles (Alkon et al., 2006; Bush et al., 2016).

The standardized SSP was 21 min and was divided into 7 three-minute episodes (e2–e8). Each episode represents a new challenging condition for the mother and her child.

In episode 2 (e2) the mother and child were alone in the laboratory room with the child exploring the new setting while the mother was sitting on a chair. In episode 3 (e3) a strange person came in and interacted with mother and child. Then, in episode 4 (e4), the mother went out of the room and the infant stayed with the strange person (separation episode). The reunion episode (e5) occurred after 30 s to 3 min (depending on the irritation and reaction of the child in front of the stranger) when the mothers were told to come back in the room and the stranger left the room. In the separation episode (e6), the mother left the room and the infant was alone. In episode 7 (e7), separation episode for the child, the stranger came back instead of the mother. Finally, in episode 8 (e8), the last reunion episode, the mother returned, while the stranger went out of the room (Table 1).

The SSP was conducted in a room with a one-way mirror to allow for the observation of the SSP and the SSP was videotaped. In the separation episodes e4, e6 and e7, the mother watched her infant in the room through the one-way mirror.

2.3. ANS measured by ECG and ICG

Two wireless lightweight mobile units (Mindware Technologies, Gahanna, USA), were used to monitor ECG and ICG simultaneously in the mother and infant. The electrodes were applied in a lead-two configuration to measure ECG waveforms and 4 impedance electrodes were applied to the neck and trunk to acquire ICG waveforms as described by Alkon et al. (2003). The electrodes were attached to leads connected to the mobile units. The mobile units were put into a small backpack for the infants to wear to minimize motion artefacts. The mothers attached their mobile units to a lap belt to hide it from the infant's view.

2.4. ANS parameters

HR is derived from the ECG waveforms and is measured as beats per minute. It can increase in response to PNS withdrawal or increasing SNS activity (Alkon et al., 2014).

RSA is an index of the PNS which is calculated using the inter beat

Table 1
Laboratory procedure (Strange Situation Procedure).

Episode 1	Lullaby; infant in mother's lap	Baseline
Episode 2	Mother and child in laboratory room; child exploring the setting	
Episode 3	Stranger coming in; interacting with mother and child	
Episode 4	Mother leaving	First separation
Episode 5	Mother coming in; stranger leaving	First reunion
Episode 6	Mother leaving	Second separation
Episode 7	Stranger instead of mother coming in	Reunion infant/ stranger
Episode 8	Mother coming in; stranger leaving	Second reunion

intervals (ECG) and respiratory rates derived from the ICG waveform at a bandwidth range of 0.15–0.80 Hz for 12-months-old (Bar-Haim et al., 2000).

PEP, an indirect inotropic measure of the SNS, refers to the time interval in milliseconds (ms) from the beginning of the ventricular depolarization (Q in ECG) until the opening of the aortic valve (B point on the ICG waveform) (Alkon et al., 2006).

The opening of the aortic valve is the onset of LVET, a chronotropic measure of the SNS, which continues until the closure of the aortic valve (X in ICG) and represents the blood ejection time of the left ventricle (ms) (Thayer and Uijtdehaage, 2001). SNS activation decreases PEP and LVET.

2.5. Data acquisition and analysis

The software BioLab 3.1 1.0J (Mindware Technologies, Gahanna, USA) was used to acquire and monitor the data. In order to address potential problems of signal interference by the physical mother-infant contact during SSP, a dZ/dt low-pass-filter was set at 10 Hz within the software before data collection. Data were extracted and scored, using HRV analysis 3.1.0F- and IMP analysis 3.1.1A-software (Mindware Technologies, Gahanna, USA), to eliminate artefacts. Therefore, each episode was divided into 6 segments of 30 s and checked visually by trained research staff. All data cleaning procedures, including surveillance at random were adapted to previously described procedures (Alkon et al., 2011; Calkins et al., 2008; Alkon et al., 2014; Waters et al., 2016; DiPietro et al., 2000). Each heart rate sub-measure was quantified as mean of the 30-second-segments.

Separation episodes sometimes were shorter than 6 segments of 30 s, because the infant was crying very much and the mother didn't want to wait outside any longer. But no episodes had to be excluded because they were too short (shorter than 2 segments).

2.6. Statistical analyses

Data were analyzed using SPSS statistical software package version 24.0 (SSP Inc., Chicago, IL). The significance level was set a priori at $p \leq 0.05$. All results are shown as mean ± standard error (SE). Normal distribution of data was tested by non-parametric Kolmogorov-Smirnov test. Since the data were normally distributed, analyses were conducted as follows: ANOVA (analysis of variance) for repeated measures was computed between the current episode and the preceding episode (e.g. e3 to e4 = e4) for mother and infant separately, for each physiological variable (HR, RSA, PEP, LVET).

Then, ANCOVA for repeated measures was calculated for each physiological variable (HR, RSA, PEP, LVET) between subjects (group: mother, child), within subjects (e1–8) as well as for interaction (episode × group). As previous research showed that boys are significantly more likely to be securely attached than girls, we supposed that male infants might react different compared to female infants (Williams and Blunk, 2003). Apart from that, other investigators reported on ANS data (HR) in mother-child-dyads during SSP with very young, adolescent mothers (Zelenko et al., 2005). This is why covariates accounted for “sex of the child” and “age of the mother at birth”.

Contrasts were computed between the current episode and the preceding episode (e.g. e2 to e3 = e3). Greenhouse-Geisser correction for repeated measures was applied.

3. Results

3.1. Effects on HR

The infants showed a significant HR increase from the baseline to the first SSP episode, e1 to e2, (F(1;90) = 27.16, $p < 0.001$, partial $\eta^2 = 0.232$), while HR significantly decreased from e2 to e3 (F(1;90) = 6.54, $p = 0.012$, partial $\eta^2 = 0.068$). A significant infant HR decrease was found in e8 compared to e7 (F(1;90) = 66.05, $p < 0.001$, partial $\eta^2 = 0.423$). On the other hand, HR increased in all three separation episodes: from e3 to e4 (F(1;90) = 21.33, $p < 0.001$, partial $\eta^2 = 0.192$), e5 to e6 (F(1;90) = 63.10, $p < 0.001$, partial $\eta^2 = 0.423$) and e6 to e7 (F(1,90) = 15.00, $p < 0.001$, partial $\eta^2 = 0.143$).

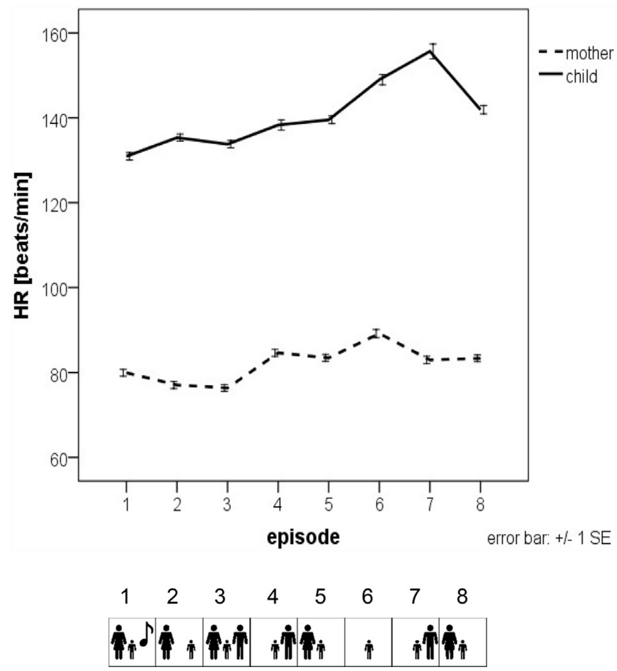

Fig. 1. Mean heart rate (HR) (± SE) of mother and infant during Strange Situation Procedure (n = 91 mother-child dyads).

Mothers showed a significant decrease in HR from e1 to e2 (F(1;90) = 26.42, $p < 0.001$, partial $\eta^2 = 0.227$), but an increase from e3 to e4 (F(1;90) = 183.95, $p < 0.001$, partial $\eta^2 = 0.671$) and e5 to e6 (F(1;90) = 87.70, $p < 0.001$, partial $\eta^2 = 0.494$). HR decreased from e6 to e7 (F(1;90) = 80.87, $p < 0.001$, partial $\eta^2 = 0.473$).

There were statistically significant episode (F(2.61; 463.68) = 4.75, $p = 0.004$, partial $\eta^2 = 0.026$) and group effects (F(1;178) = 1467.92, $p < 0.001$, partial $\eta^2 = 0.892$), as well as a significant group-by-episode effect (F (2.61;463.68) = 37.11, $p < 0.001$, partial $\eta^2 = 0.172$). Contrasts showed that this group-by-episode effect was detectable from e5 to e6 (F(1;178) = 10.29; $p = 0.002$, partial $\eta^2 = 0.055$), e6 to e7 (F(1;178) = 46.32, $p < 0.001$, partial $\eta^2 = 0.206$) and from e7 to e8 (F(1;178) = 65.13, $p < 0.001$, partial $\eta^2 = 0.268$) (Fig. 1).

None of the covariates (“sex of the child”, “age of the mother at birth”) influenced the results for HR.

3.2. Effects on RSA

The infant's RSA measures during SSP showed a statistically significant RSA increase from e7 to e8 (F(1;90) = 23.81, $p < 0.001$, partial $\eta^2 = 0.209$), as well as a significant decrease from e3 to e4 (F(1;90) = 6.12, $p = 0.015$, partial $\eta^2 = 0.064$) and e6 to e7 (F(1;90) = 9.19, $p = 0.003$, partial $\eta^2 = 0.093$). Mother's RSA increased significantly from e1 to e2 (F(1;90) = 50.998, $p < 0.001$, partial $\eta^2 = 0.362$), decreased from e2 to e3 (F(1;90) = 10.58, $p = 0.002$, partial $\eta^2 = 0.105$) and increased from e7 to e8 (F(1;90) = 7.69, $p = 0.007$, partial $\eta^2 = 0.079$). On the other hand, maternal RSA decreased significantly in e3–e4 (F(1;90) = 1467.92, $p < 0.001$, partial $\eta^2 = 0.266$) and remained almost unaffected from e6 to e7 (F(1;90) = 0.22, $p = 0.638$, partial $\eta^2 = 0.002$).

There was no significant main episode effect for RSA (F(4.43;787.72) = 0.87, $p = 0.492$, partial $\eta^2 = 0.005$). However, there was a significant main RSA group effect (infant vs. mother) (F

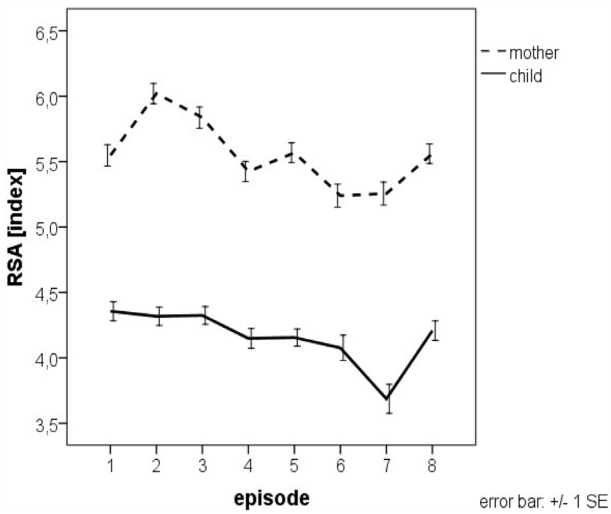

Fig. 2. Mean respiratory sinus arrhythmia (RSA) (± SE) of mother and infant during Strange Situation Procedure (n = 91 mother-child dyads).

(1;178) = 168.60, $p < 0.001$, partial $\eta^2 = 0.486$) as well as a significant group-by-episode effect (F(4.43;787.72) = 3.47, p = 0.006, partial $\eta^2 = 0.019$). Contrast analyses showed that a significant group-by-episode effect for RSA was detectable from e1 to e2 (F (1;178) = 21.07, $p < 0.001$, partial $\eta^2 = 0.105$), e2 to e3 (F (1;178) = 4.73, p = 0.031, partial $\eta^2 = 0.026$), e7 to e8 (F (1;178) = 6.84, p = 0.010, partial $\eta^2 = 0.037$). From e6 to e7 (F (1;178) = 5.24, p = 0.023, partial $\eta^2 = 0.029$), infant's RSA decreased, while mother's RSA was nearly stable (Fig. 2).

None of the covariates (“sex of the child”, “age of the mother at birth”) influenced the results for RSA.

3.3. Effects on LVET

Infant's LVET measures during SSP revealed a statistically significant decrease from e1 to e2 (F(1;90) = 8.43, p = 0.005, partial $\eta^2 = 0.086$), showing an increase in SNS activation. From e7 to e8 there was a significant LVET increase (F(1;90) = 67.56, $p < 0.001$, partial $\eta^2 = 0.429$). From e3 to e4 (F(1;90) = 14.76, $p < 0.001$, partial $\eta^2 = 0.141$), e5 to e6 (F(1;90) = 63.13, $p < 0.001$, partial $\eta^2 = 0.412$) and e6 to e7 (F(1;90) = 17.64, $p < 0.001$, partial $\eta^2 = 0.164$), there was a significant infant LVET decrease (SNS activation).

Mother's LVET increased significantly from e1 to e2 (F (1;90) = 4.86, p = 0.030, partial $\eta^2 = 0.051$) and from e4 to e5 (F (1;90) = 6.47, p = 0.013, partial $\eta^2 = 0.067$). It decreased significantly from e3 to e4 (F(1;90) = 27.08, $p < 0.001$, partial $\eta^2 = 0.231$) and e5 to e6 (F(1;90) = 15.15, $p < 0.001$, partial $\eta^2 = 0.144$), but increased significantly from e6 to e7 (F(1;90) = 11.19, $p < 0.001$, partial $\eta^2 = 0.111$).

There were statistically significant main effects for episode (F (3.67;653.60) = 2.99, p = 0.021, partial $\eta^2 = 0.017$), group (F (1;178) = 796.63, $p < 0.001$, partial $\eta^2 = 0.817$) as well as for group-by-episode (F(3.67;653.60) = 19.74, $p < 0.001$, partial $\eta^2 = 0.100$). Contrasts showed significant group-by-episode effects from e1 to e2 (F (1;178) = 12.29, p = 0.001, partial $\eta^2 = 0.065$), e7 to e8 (F (1;178) = 37.97, $p < 0.001$, partial $\eta^2 = 0.176$), e5 to e6 (F (1;178) = 5.05, p = 0.026, partial $\eta^2 = 0.028$) and e6 to e7 (F (1;178) = 28.3, $p < 0.001$, partial $\eta^2 = 0.137$). Infant LVET decreased significantly more from e5 to e6 and increased significantly more from e7 to e8. From e6 to e7 infant's LVET decreased while maternal LVET increased (Fig. 3).

None of the covariates (“sex of the child”, “age of the mother at birth”) influenced the results for LVET.

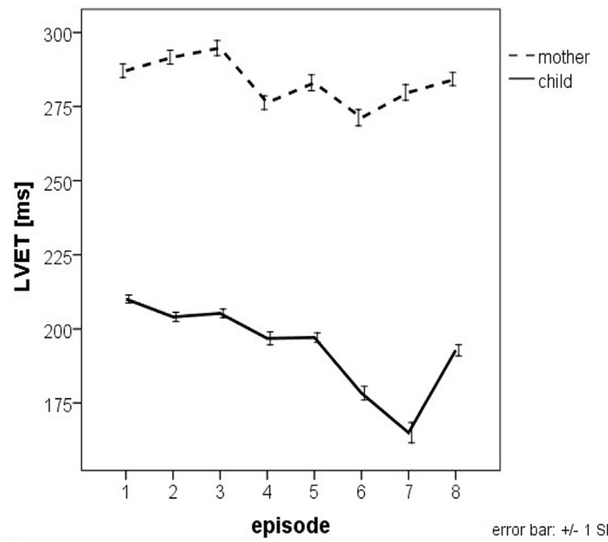

Fig. 3. Mean left ventricular ejection time (LVET) (± SE) of mother and infant during Strange Situation Procedure (n = 91 mother-child dyads).

3.4. Effects on PEP

The infants showed no significant PEP changes across the SSP episodes. On the other hand, mothers showed significant decrease in PEP (SNS activation) from e1 to e2 (F(1;90) = 13.76, $p < 0.001$, partial $\eta^2 = 0.133$), e7 to e8 (F(1;90) = 32.86, $p < 0.001$, partial $\eta^2 = 0.267$) and e4 to e5 (F(1;90) = 45.14, $p < 0.001$, partial $\eta^2 = 0.334$) .

Mothers´ PEP significantly increased from e2 to e3 (F (1;90) = 23.39, $p < 0.001$, partial $\eta^2 = 0.206$) and from e6 to e7 (F (1;90) = 31.04, $p < 0.001$, partial $\eta^2 = 0.256$), showing no SNS activation.

There was no significant main episode effect (F(5.86; 1043.03) = 0.59, p = 0.733, partial $\eta^2 = 0.003$). However, a significant main group effect (F(1;178) = 1335.06, $p < 0.001$, partial $\eta^2 = 0.882$) as well as a significant group-by-episode effect for PEP were found (F(5.86; 1043.03) = 7.84, $p < 0.001$, partial $\eta^2 = 0.042$). Contrast analyses revealed significant group-by-episode differences between maternal and infant's PEP from e1 to e2 (F(1;178) = 4.94, p = 0.028, partial $\eta^2 = 0.027$), e2 to e3 (F(1;178) = 18.13, $p < 0.001$, partial $\eta^2 = 0.092$), e4 to e5 (F(1;178) = 19.91, $p < 0.001$, partial $\eta^2 = 0.101$), e7 to e8 (F(1;178) = 15.72, $p < 0.001$, partial $\eta^2 = 0.081$) and e6 to e7 (F(1;178) = 14.60, $p < 0.001$, partial $\eta^2 = 0.076$). Mothers showed significantly higher SNS activation from e1 to e2, e4 to e5 and e7 to e8 than infants. Maternal PEP increases significantly from e6 to e7 compared to infant's PEP (Fig. 4).

None of the covariates (“sex of the child”, “age of the mother at birth”) influenced the results for PEP.

4. Discussion

Simultaneous measures of mothers and their 12-month-old infants are feasible and showed some significant responses to the SSP episodes. However, the infant ANS system seems to be more sensitive to the unexpected contact to a strange person instead of the mother (e7).

The infants showed a significant PNS withdrawal during that episode compared to the previous episode. Also, the measure of LVET in our experimental setting was more sensitive than PEP in showing SNS activation during e7 in the one-year old infants but not in the mothers.

Our general findings confirm results of other groups that showed, that the SSP paradigm induces stress in mothers and infants with measurable changes in PNS and SNS (Zelenko et al., 2005; Hill-Soderlund et al., 2008; Oosterman and Schuengel, 2007a; Smith et al., 2016). The SSP represents a mild stress situation (Oosterman and

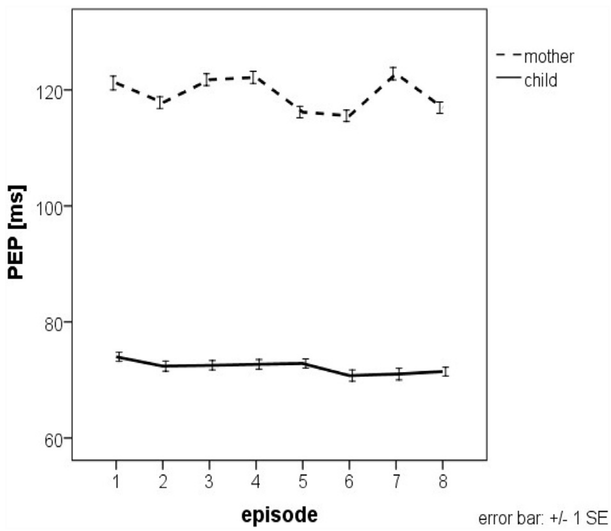

Fig. 4. Mean pre-ejection period (PEP) (± SE) of mother and infant during Strange Situation Procedure (n = 91 mother-child dyads).

Schuengel, 2007a; Ainsworth, 1978), that should theoretically be regulated by parasympathetic withdrawal (increase in HR, decrease in RSA), whereas intense challenges are followed by SNS activation (increase in HR, decrease in PEP and LVET). We know that PNS is a key indicator of self-regulation, with links to numerous developmental outcomes (Smith et al., 2016). The continuous decrease in infant RSA during SSP reflects PNS withdrawal due to increase in the infants' attachment-related stress activation (Hill-Soderlund et al., 2008; Bar-Haim et al., 2000; Bazhenova et al., 2001).

Oosterman has measured PEP in young foster children (2–7 years old), without finding significant PEP changes during a modified SSP. The explanation for these findings was that the SSP is not threatening enough to activate the SNS, and the documented HR-changes are thus due to PNS withdrawal (Oosterman and Schuengel, 2007a, 2007b). However, our data indicate that the well-documented continuous HR increases due to SSP in the children results not only from vagal withdrawal, but also from a successive increase in the infants' SNS activity, reflected by the LVET. Previous research investigated infant PEP as the SNS parameter and not LVET (Oosterman and Schuengel, 2007b; Oosterman et al., 2010). Maternal PEP and infant and maternal LVET have not yet been investigated in young children or mother-child dyads during a stress-paradigm. LVET is supposed to be a useful measure of sympathetic influences (Thayer and Uijtdehaage, 2001).

4.1. Mother-infant-interactions from e1 to e2

We found significant differences between maternal and infant ANS reactions from e1 to e2 for all parameters. Episode 2 is the beginning of the standardized SSP, when mother and infant enter the strange room after a baseline episode Infants showed significant HR increases and LVET decreases, reflecting increases in sympathetic activity, probably due to the end of the lullaby and to the unknown, novelty of the laboratory room. Mothers, on the other hand, seemed to “relax”, as shown by their decreases in HR and increases in RSA as the SSP starts. Unfortunately, these results cannot be compared to previous literature, since the SSP usually does not start before e2.

4.2. Mother-infant-interactions during reunion in e5 and e8

E8, the last reunion, is supposed to be the most stressful episode for infants (Hill-Soderlund et al., 2008). Concerning the reunion episodes e5 and e8, we found e8, the final mother-infant reunion, to reveal significant ANS reactions in the infants compared to e5. Infant HR findings reported by Zelenko et al. (2005) are consistent with our results of infants' HR decreases when reunited with the mother in e8. Our results showed that this stress reduction resulted from a sympathetic withdrawal (LVET increase) and a parasympathetic increase (RSA increase). The significant RSA increase in e8 for mothers and infants in our findings are consistent with findings of Hill-Soderlund, who also described RSA increase in mother and infants in e8 (Hill-Soderlund et al., 2008).

Concerning the SNS responsivity during e8, infants were regulating by not activating their SNS, visible in LVET increase, while infant PEP showed no significant change from e7. This is consistent with previous findings showing no significant PEP changes during modified SSP for children (3–6//2–7 years) (Oosterman and Schuengel, 2007a, 2007b).

Mothers on the other hand revealed significant PEP changes from e7 to e8, with an increase in PEP showing no sympathetic activation during e8. To our knowledge, neither maternal PEP nor maternal LVET have been investigated during separation episodes in SSP.

Our findings underline previous research identifying e8 (second reunion) to induce more pronounced ANS reactions than e5 (first reunion) (Oosterman and Schuengel, 2007a).

In addition, we found significant differences between maternal and infant ANS reactions for all parameters from e7 to e8 and, on the other hand, only for PEP from e4 to e5. The clear interaction effect in e8 is interesting when compared to Zelenko's study who found more parallelism between maternal and infant HR in secure attached mother-child dyads than in insecure attached dyads (Zelenko et al., 2005). It is noteworthy that Zelenko did not measure PNS or SNS measures.

4.3. Mother-infant-interactions during separation in e4 and e6

SSP induced a significant HR increase for mothers and infants from e3 to e4 and from e5 to e6, with a significant increase in mothers. Both, maternal and infant HR increased with a more pronounced infant HR increase in e6, triggered by the fact that the mother left the infant all alone in the room. Previous researchers also found HR increase in mothers and infants in e4 and e6 (Zelenko et al., 2005).

Concerning RSA, we found significant differences between maternal and infant RSA from e6 to e7, but not in e4 and e6. While in both episodes, e4 and e6, we found an RSA decrease, which means a decreasing parasympathetic activity. Our findings are supported by other researchers, who described RSA decrease in e4 and e6 for mother and infant (Hill-Soderlund et al., 2008; Smith et al., 2016; Oosterman and Schuengel, 2007b; Oosterman et al., 2010). Mother's and infant's LVET decreased in e4 and e6, indicating an increase in SNS activity, whenever the mother left the room. In e6 we found a significant difference between maternal and infant LVET with SNS increase, more pronounced in infants. Maternal and infant PEP were nearly stable in e4 and e6 compared to the preceding episode. The HR increase in these episodes seems not to account for PEP decrease. This was also found by Oosterman (Oosterman and Schuengel, 2007a).

In summary, our results indicate, that chronotropic SNS responses, reflected by the decreased LVET, mainly account for the increase in HR in both, mother and child, during separation in e4 and e6. LVET was measured successfully before and validated as SNS measure (Thayer and Uijtdehaage, 2001; Cokkinos et al., 1976), but not yet in mother-child dyads during SSP.

4.4. E7: the underestimated separation episode

In e7 (e7–e6) we found significant differences in ANS regulation between mother and infant for all parameters with clear opposite effects for maternal and infant HR and LVET. This is an important result, since attachment related research generally focuses on reunion episodes (e5, e8) or separation episodes (e4, e6) and not on e7 (Benoit, 2004; Smith et al., 2016). Furthermore, attunement or synchrony and not opposite ANS activation between caregiver and infant is supposed to be

Table 2
a, b Descriptive data [mean] and contrasts [p] from e6 to e7 in mothers and infants separately (n = 91 mother-child dyads).

	e6 [mean]	e6 [SE]	e7 [mean]	e7 [SE]	p
2a: mothers					
HR [beats/min]	89.6	11.4	82.6	11.0	< 0.000
RSA [index]	5.3	1.0	5.3	1.1	0.638
LVET [ms]	271.6	36.0	282.6	29.4	0.001
PEP [ms]	117.5	11.9	125.4	13.6	< 0.000
2b: infants					
HR [beats/min]	149.8	15.6	157.7	24.6	< 0.000
RSA [index]	4.0	1.3	3.4	1.4	0.003
LVET [ms]	175.3	28.3	160.9	48.5	< 0.000
PEP [ms]	72.2	12.5	72.7	11.9	0.721

Abbreviation: epi, episode; HR, heart rate; LVET, left ventricular ejection time; min, minute; ms, millisecond; p, level of significance; PEP, pre-ejection period; RSA, respiratory sinus arrhythmia; SE, standard error; SSP, Strange Situation Procedure.

a natural process (Hill-Soderlund et al., 2008). Zelenko showed in this context higher physiological attunement for HR in secure attached dyads compared to insecure-resistant dyads (Zelenko et al., 2005).

The presence of an unexpected stranger induced HR increase in the infants and HR decreases in their mothers compared to the proceeding episode (e6) (Table 2a/b). The mother, who watched the episode from outside the room through a window, showed a stress reduction response probably because her infant was no longer left alone. In contrast to that, infant's HR increased to a maximum HR in response to the stranger, coming into the room instead of the mother. The strange, unexpected person possibly augments stress in addition to the distress produced by the fact that the mother left the room in e6.

To our knowledge, there was one study that explored maternal and infant physiological reaction simultaneously during SSP in 1985: Donovan and Leavitt reported HR-changes by comparing securely- and insecure-attached mother-child dyads. Securely-attached children showed a HR decrease in contact to the strange person in e7 (Donovan and Leavitt, 1985), which is contrary to our findings. After that, it was Zelenko, who investigated in 2005 mothers and their one-year-old infants concerning HR changes during SSP, but he concentrated on the differentiation between attachment groups and missed out e7 (Zelenko et al., 2005). In 2007 Oosterman elicited e7 in preschool foster children in a modified SSP, showing a HR decrease in this episode (Oosterman and Schuengel, 2007a). These results contrast our findings. However, as the infants in our study are younger, non-foster infants, and Oosterman's SSP is modified, our results are difficult to compare.

In order to understand which ANS branch (SNS or PNS) induces maternal HR reduction and infant HR increase in e7 compared to e6, we investigated RSA. A significant RSA interaction effect between mother and infant was observed in contact to the unexpected stranger (e6–e7), with infants showing a PNS withdrawal, while mothers RSA was nearly stable (Table 2a, b). Our infant results confirm Stifter, who showed that within a sample of healthy 4–5-year-old children, 40% reacted with a RSA decrease in response to an unknown person asking questions (Stifter et al., 2011). It is well known that the awareness of safety increases PNS activity (Oosterman and Schuengel, 2007b; Porges, 2003). Therefore, we interpret the maternal RSA stability in e7 in the way that mother's perception of safety was not affected from e6 to e7. This maternal reaction may be supported by the fact that mothers are watching the stranger-infant interaction through a window, which intensifies the feeling of control and consecutively reduces anxiety.

Our findings are different than Hill-Soderlund's, who reported an increase in infant's mean RSA and a decrease in mother's mean RSA in e7 in mother-child dyads of one-year-old infants (Hill-Soderlund et al., 2008). The results are difficult to compare because of the different sample characteristics and that Hill-Soderlund did not measure SNS changes (PEP or LVET) or HR variations. Apart from that, the emphasis was on the differences between secure attached and insecure-avoidant attached children and their mothers, excluding insecure-resistant attached children (n = 15).

In 2007, Oosterman elicited e7 in preschool-age foster children, showing a contrast to our results with an increase in RSA during this episode (Oosterman and Schuengel, 2007a). However, as the children in our sample are younger and living with their biological parents, the results are difficult to compare.

Concerning PEP, we found a significant interaction effect between mother and infant in e7. Infants showed no significant PEP changes all over the SSP. This confirms Oosterman, who described the absence of significant PEP-effects in preschool-age children (3–6 years) in a modified SSP (Oosterman and Schuengel, 2007b). This finding might suggest that infants are regulating stress, induced by SSP, mainly by PNS withdrawal. Former research thus supposed SSP to be not threatening enough to activate the SNS (Oosterman and Schuengel, 2007b). We, however found infants regulating e7 by LVET decrease, which means SNS activation. If recognizing the contact to the unexpected stranger as a challenge or threat, this SNS activation is a healthy reaction of the infant, according to previous research, mentioning “defense response”, reflected by SNS activation and PNS withdrawal (Cannon, 1914; McKlveen et al., 2015; McCorry, 2007). We thus assume that in our sample in e7, the strange person provokes strong and healthy PNS decrease and SNS increase reactions in one-year old infants of mothers without own CM experiences (Table 2a, b).

Mothers on the other hand showed a LVET increase and so an opposite LVET effect leading to a significant interaction effect between mother and infant from e6 to e7. Additional to LVET, mothers are regulating this episode by an increasing PEP (Table 2a, b). As maternal RSA is nearly stable during e7, Increases in LVET and PEP (SNS responsivity) are responsible for decreases in HR for the mothers in our study. Maternal PEP and LVET have not yet been investigated in the SSP context.

We could thus show that the change from e6 to e7 in the SSP is rather supposed to be a mild stressor, is regulated by SNS downregulation in mothers, resulting in decreases in maternal HR. Furthermore, infants with PNS withdrawal and increasing SNS activity via LVET could be measured, explaining infants HR increases.

4.5. LVET as the chronotropic index of the SNS

Systolic time intervals are known to be sensitive to changes during challenging situations (McCubbin et al., 1983) and PEP and LVET decrease in response to stress (Brindle et al., 2014; Cacioppo et al., 1994; McCubbin et al., 1983; Yim et al., 2015). Generally, there is only little research done on PEP or LVET measures in young children (Bush et al., 2016; van Dijk et al., 2013; Alkon et al., 2003). PEP has been identified as an inotropic index reflecting changes in contractility during stress whereas LVET, as a chronotropic index, enables to measure sympathetic chronotropic influences (Thayer and Uijtdehaage, 2001). In our study, we measured both, the PEP and the LVET, in order to distinguish between the inotropic and the chronotropic reactivity to attachment related stress. By comparing both measures, infants showed a main chronotropic response of the SNS via the LVET without any inotropic reactivity due to separation and reunion. Others confirm the lack of a PEP response due to SSP procedure and conclude that separation may not be threatening enough to activate the SNS (Oosterman and Schuengel, 2007a). In contrast to infant's LVET, infant PEP does not show any variability all over the SSP. Oosterman et al. (2010) described stable PEP values for secure attached children, in contrast to unsecure ones who showed higher variability in PEP values. PEP has been considered as the main measure of the SNS and has been widely used in attachment, emotional and cognitive-related measures of the ANS (Oosterman and Schuengel, 2007a, 2007b). Though, PEP has been shown to be minimally affected by HR and to be a robust marker of stroke volume with the index of PEP/LVET being a sensitive index of cardiac function (Cokkinos et al., 1976). PEP alterations become relevant with growing age due to the fact that stress reactivity in child is mainly frequency related and in adult persons contractility related. These findings are confirmed by our data showing unaltered PEP due to SSP in the one-year-old infants, whereas mothers have significant inotropic reactions during SSP. Published LVET measures in children are rare and come from echocardiographic studies, showing that systolic time intervals and HR are closely correlated but less important than in adults and that only the PEP/LVET ratio was measured as independent from HR without any sex differences (Vitolo et al., 1991). A significant influence of sex on LVET was shown in elderly subjects (60 years–90 years) (Willems et al., 1970). Beside, other authors could show significant LVET withdrawal in adults during fear inducing films (Kreibig et al., 2007).

Comparison of children, adolescents and adults showed a distinct tendency toward lengthening of the PEP and LVET with increasing age. This lengthening was independent of changes in HR and was more pronounced for PEP than for LVET (Wanderman et al., 1981). For our sample, mean LVET was 0.21 ms in the baseline episode (e1) which is comparable to previous literature describing normal standards of LVET in children (Cantor et al., 1978). Our data reveal RSA values for one-year-olds with a maximum index of 4.3, whereas RSA in 3–5-years-old have been determined between 4.7 and 6.0 (Smith et al., 2016) indicating that RSA also increases with increasing age of the child (Alkon et al., 2006).

LVET in our study reflects infant chronotropic SNS reactivity and therefore might be a more sensitive SNS marker for stress in young children compared to PEP, that, in our data, didn't show significant variability during the SSP. Our data indicate that both, the PNS and the chronotropic SNS system, are responsible for HR changes during attachment-sensitive stress-regulation in the one-year-old infant.

5. Limitations

Limitations of this study were data acquisition problems due to child movement and physical contact between mother and infant followed by electrode disturbance. Apart from that, data acquisition showed disturbances due to procedure faults. This explains the comparatively small sample size of mothers without CM experience with complete data set (n = 91).

Apart from that, the demographic characteristics (mothers with a comparatively high level of education) and geographic location of the sample limits the study's generalizability.

Furthermore, there were a high percentage of mothers suffering from chronical diseases. This has to be considered when comparing our results with other studies with populations with different demographic characteristics.

6. Conclusion

In summary, continuous, simultaneous, non-invasive PNS and SNS measures in mothers and their 12-months-old infants during the SSP are feasible. By using this technique, we were able to identify the LVET as a chronotropic measure for the characterization of the SNS branch of the ANS in 1-year-old infants. Vagal withdrawal and increase in SNS activity are detectable in both, mother and infant, however, at different time points during the SSP. As expected, the most stressful moment for mothers is, when their infants are left alone. The most stressful episode for infants, however, is to come in contact with a stranger in the moment when the mother is eagerly awaited.

Ethical considerations

This article reports the results of experimental investigation with human subjects.

The work described has been carried out in accordance with "The Code of Ethics of the World Medical Association" (Declaration of Helsinki).

We confirm to have obtained written consent and assent from the subjects, as appropriate. The privacy rights have always been observed.

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Declaration of competing interest

The authors have no competing interests to declare.

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References

Ainsworth, M.D.S., 1978. Patterns of Attachment: A Psychological Study of the Strange Situation. Lawrence Erlbaum Associates.

Alkon, A., Goldstein, L.H., Smider, N., Essex, M.J., Kupfer, D.J., Boyce, W.T., Ablow, J.C., Alkon, A., Armstrong, J.M., Harrington, R., Kraemer, H.C., Measelle, J.R., Nelson, C.A., Quas, J., Steinberg, L., 2003. Developmental and contextual influences on autonomic reactivity in young children. Dev. Psychobiol. 42, 64–78. https://doi.org/10.1002/dev.10082.

Alkon, A., Lippert, S., Vujan, N., Rodriquez, M.E., Boyce, W.T., Eskenazi, B., 2006. The ontogeny of autonomic measures in 6- and 12-month-old infants. Dev. Psychobiol. 48, 197–208. https://doi.org/10.1002/dev.20129.

Alkon, A., Boyce, W.T., Davis, N.V., Eskenazi, B., 2011. Developmental changes in autonomic nervous system resting and reactivity measures in Latino children from 6 to 60 months of age. J. Dev. Behav. Pediatr. 32, 668–677. https://doi.org/10.1097/DBP.0b013e3182331fa6.

Alkon, A., Harley, K.G., Neilands, T.B., Tambellini, K., Lustig, R.H., Boyce, W.T., Eskenazi, B., 2014. Latino children's body mass index at 2–3.5 years predicts sympathetic nervous system activity at 5 years. Child. Obes. 10, 214–224. https://doi.org/10.1089/chi.2013.0063.

Bader, K., Hänny, C., Schäfer, V., Neuckel, A., Kuhl, C., 2009. Childhood Trauma Questionnaire - Psychometrische Eigenschaften einer deutschsprachigen Version. Zeitschrift für Klinische Psychologie und Psychotherapie 38(4), 223–230.

Bar-Haim, Y., Marshall, P.J., Fox, N.A., 2000. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Dev. Psychobiol. 37, 44–56.

Bazhenova, O.V., Plonskaia, O., Porges, S.W., et al., 2001. Vagal reactivity and affective adjustment in infants during iteraction challenges. Child Dev. 72, 1314–1326. https://doi.org/10.1111/1467-8624.00350.

Benoit, D., 2004. Infant-parent attachment: definition, types, antecedents, measurement and outcome. Paediatr. Child Health 9, 541–545.

Berntson, G.G., Cacioppo, J.T., Grossman, P., 2007. Whither vagal tone. Biol. Psychol. 74, 295–300. https://doi.org/10.1016/j.biopsycho.2006.08.006.

Blair, C., Berry, D., Mills-Koonce, R., Granger, D., Investigators, F.L.P., 2013. Cumulative effects of early poverty on cortisol in young children: moderation by autonomic nervous system activity. Psychoneuroendocrinology 38, 2666–2675. https://doi.org/10.1016/j.psyneuen.2013.06.025.

Bowers, M.E., Yehuda, R., 2016. Intergenerational transmission of stress in humans. Neuropsychopharmacology 41, 232–244. https://doi.org/10.1038/npp.2015.247.

Bowlby, J., 1980. Attachment and Loss Loss Sadness and Depression.

Bowlby, J., 1982. Attachment and Loss. Basic Books.

Brindle, R.C., Ginty, A.T., Phillips, A.C., Carroll, D., 2014. A tale of two mechanisms: a meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. Psychophysiology 51, 964–976. https://doi.org/10.1111/psyp.12248.

Bush, N.R., Caron, Z.K., Blackburn, K.S., Alkon, A., 2016. Measuring cardiac autonomic nervous system (ANS) activity in toddlers - resting and developmental challenges. J. Vis. Exp. https://doi.org/10.3791/53652.

Cacioppo, J.T., Berntson, G.G., Binkley, P.F., Quigley, K.S., Uchino, B.N., Fieldstone, A., 1994. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. Psychophysiology 31, 586–598.

Calkins, S.D., Graziano, P.A., Berdan, L.E., Keane, S.P., Degnan, K.A., 2008. Predicting cardiac vagal regulation in early childhood from maternal-child relationship quality during toddlerhood. Dev. Psychobiol. 50, 751–766. https://doi.org/10.1002/dev.20344.

Cannon, W.B., 1914. The emergency function of the adrenal medulla in pain and major emotions. Am. J. Phys. 3, 356–372.

Cantor, J.R., Zillmann, D., Day, K.D., 1978. Relationship between cardiorespiratory fitness and physiological responses to films. Percept. Mot. Skills 46, 1123–1130.

https://doi.org/10.2466/pms.1978.46.3c.1123.
Cassidy, J., Shaver, P.R., 1999. Handbook of Attachment: Theory, Research, and Clinical Applications. Guilford Press.
Chambers, J., 2017. The neurobiology of attachment: from infancy to clinical outcomes. Psychodyn. Psychiatry 45, 542–563. https://doi.org/10.1521/pdps.2017.45.4.542.
Cokkinos, D.V., Heimonas, E.T., Demopoulos, J.N., Harralambakis, A., Tsartsalis, G., Gardikas, C.D., 1976. Influence of heart rate increase on uncorrected pre-ejection period/left ventricular ejection time (PEP/LVET) ratio in normal individuals. Br. Heart J. 38, 683–688.
Critchley, H.D., Nagai, Y., Gray, M.A., Mathias, C.J., 2011. Dissecting axes of autonomic control in humans: insights from neuroimaging. Auton. Neurosci. 161, 34–42. https://doi.org/10.1016/j.autneu.2010.09.005.
Crittenden, P.M., 2017. Gifts from Mary Ainsworth and John Bowlby. Clin. Child Psychol. Psychiatry 22, 436–442. https://doi.org/10.1177/1359104517716214.
DiPietro, J.A., Costigan, K.A., Pressman, E.K., Doussard-Roosevelt, J.A., 2000. Antenatal origins of individual differences in heart rate. Dev. Psychobiol. 37, 221–228.
Donovan, W.L., Leavitt, L.A., 1985. Physiologic assessment of mother-infant attachment. J. Am. Acad. Child Psychiatry 24, 65–70.
Feldman, R., 2012. Parent-infant synchrony: a biobehavioral model of mutual influences in the formation of affiliative bonds. Monogr. Soc. Res. Child Dev. 77, 42–51. https://doi.org/10.1111/j.1540-5834.2011.00660.x.
Feldman, R., Eidelman, A.I., 2007. Maternal postpartum behavior and the emergence of infant-mother and infant-father synchrony in preterm and full-term infants: the role of neonatal vagal tone. Dev. Psychobiol. 49, 290–302. https://doi.org/10.1002/dev.20220.
Feldman, J.L., Ellenberger, H.H., 1988. Central coordination of respiratory and cardiovascular control in mammals. Annu. Rev. Physiol. 50, 593–606. https://doi.org/10.1146/annurev.ph.50.030188.003113.
Garfinkel, S.N., Zorab, E., Navaratnam, N., Engels, M., Mallorquí-Bagué, N., Minati, L., Dowell, N.G., Brosschot, J.F., Thayer, J.F., Critchley, H.D., 2016. Anger in brain and body: the neural and physiological perturbation of decision-making by emotion. Soc. Cogn. Affect. Neurosci. 11, 150–158. https://doi.org/10.1093/scan/nsv099.
Hill-Soderlund, A.L., Mills-Koonce, W.R., Propper, C., Calkins, S.D., Granger, D.A., Moore, G.A., Gariepy, J.-L., Cox, M.J., 2008. Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. Dev. Psychobiol. 50, 361–376. https://doi.org/10.1002/dev.20302.
Hofer, M.A., 2006. Psychobiological roots of early attachment. Curr. Dir. Psychol. Sci. 15, 84–88. https://doi.org/10.1111/j.0963-7214.2006.00412.x.
Kreibig, S.D., Wilhelm, F.H., Roth, W.T., Gross, J.J., 2007. Cardiovascular, electrodermal, and respiratory response patterns to fear- and sadness-inducing films. Psychophysiology 44, 787–806. https://doi.org/10.1111/j.1469-8986.2007.00550.x.
Lewis, M., Haviland-Jones, J.M., Barrett, L.F., 2008. Handbook of Emotions. Guilford Press.
Manini, B., Cardone, D., Ebisch, S.J.H., Bafunno, D., Aureli, T., Merla, A., 2013. Mom feels what her child feels: thermal signatures of vicarious autonomic response while watching children in a stressful situation. Front. Hum. Neurosci. 7, 299. https://doi.org/10.3389/fnhum.2013.00299.
McCorry, L.K., 2007. Physiology of the autonomic nervous system. Am. J. Pharm. Educ. 71, 78.
McCubbin, J.A., Richardson, J.E., Langer, A.W., Kizer, J.S., Obrist, P.A., 1983. Sympathetic neuronal function and left ventricular performance during behavioral stress in humans: the relationship between plasma catecholamines and systolic time intervals. Psychophysiology 20, 102–110.
McKlveen, J.M., Myers, B., Herman, J.P., 2015. The medial prefrontal cortex: coordinator of autonomic, neuroendocrine and behavioural responses to stress. J. Neuroendocrinol. 27, 446–456. https://doi.org/10.1111/jne.12272.
Michels, N., Sioen, I., Clays, E., De Buyzere, M., Ahrens, W., Huybrechts, I., Vanaelst, B., De Henauw, S., 2013. Children's heart rate variability as stress indicator: association with reported stress and cortisol. Biol. Psychol. 94, 433–440. https://doi.org/10.1016/j.biopsycho.2013.08.005.
Oosterman, M., Schuengel, C., 2007a. Physiological effects of separation and reunion in relation to attachment and temperament in young children. Dev. Psychobiol. 49, 119–128. https://doi.org/10.1002/dev.20207.
Oosterman, M., Schuengel, C., 2007b. Autonomic reactivity of children to separation and reunion with foster parents. J. Am. Acad. Child Adolesc. Psychiatry 46, 1196–1203. https://doi.org/10.1097/chi.0b013e3180ca839f.
Oosterman, M., De Schipper, J.C., Fisher, P., Dozier, M., Schuengel, C., 2010. Autonomic reactivity in relation to attachment and early adversity among foster children. Dev. Psychopathol. 22, 109. https://doi.org/10.1017/S0954579409990290.
Porges, S.W., 2003. Social engagement and attachment: a phylogenetic perspective. Ann. N. Y. Acad. Sci. 1008, 31–47.
Porges, S.W., 2007. The Polyvagal Perspective. Biol. Psychol. 74(2), 116–143.
Smith, J.D., Woodhouse, S.S., Clark, C.A.C., Skowron, E.A., 2016. Attachment status and mother-preschooler parasympathetic response to the strange situation procedure. Biol. Psychol. 114, 39–48. https://doi.org/10.1016/j.biopsycho.2015.12.008.
Spangler, G., Grossmann, K.E., 1993. Biobehavioral organization in securely and insecurely attached infants. Child Dev. 64, 1439–1450.
Sroufe, L.A.E., 1977. Heart Rate as a Covergent Measure in Clinical and Developmental Research. Merrill. Palmer. Q.
Sroufe, L.A., 1997. Psychopathology as an outcome of development. Dev. Psychopathol. 9, 251–268.
Stifter, C.A., Dollar, J.M., Cipriano, E.A., 2011. Temperament and emotion regulation: the role of autonomic nervous system reactivity. Dev. Psychobiol. 53, 266–279. https://doi.org/10.1002/dev.20519.
Swain, J.E., Lorberbaum, J.P., Kose, S., Strathearn, L., 2007. Brain basis of early parent–infant interactions: psychology, physiology, and in vivo functional neuroimaging studies. J. Child Psychol. Psychiatry 48, 262–287. https://doi.org/10.1111/j.1469-7610.2007.01731.x.
Thayer, J.F., Uijtdehaage, S.H., 2001. Derivation of chronotropic indices of autonomic nervous system activity using impedance cardiography. Biomed. Sci. Instrum. 37, 331–336.
van Dijk, A.E., van Lien, R., van Eijsden, M., Gemke, R.J.B.J., Vrijkotte, T.G.M., de Geus, E.J., 2013. Measuring cardiac autonomic nervous system (ANS) activity in children. J. Vis. Exp. https://doi.org/10.3791/50073.
Van Rosmalen, L., Van der Veer, R., Van der Horst, F., 2015. Ainsworth's strange situation procedure: the origin of an instrument. J. Hist. Behav. Sci. 51, 261–284. https://doi.org/10.1002/jhbs.21729.
Vitolo, E., Colombo, A., Castini, D., Morabito, A., 1991. Evaluation of systolic time intervals in a group of healthy children 10–12 years old. Acta Cardiol. 46, 631–640.
Wanderman, K.L., Hayek, Z., Ovsyshcher, I., Loutaty, G., Cantor, A., Gussarsky, Y., Gueron, M., 1981. Systolic time intervals in adolescents. Normal standards for clinical use and comparison with children and adults. Circulation 63, 204–209.
Waters, S.F., Boyce, W.T., Eskenazi, B., Alkon, A., 2016. The impact of maternal depression and overcrowded housing on associations between autonomic nervous system reactivity and externalizing behavior problems in vulnerable Latino children. Psychophysiology 53, 97–104. https://doi.org/10.1111/psyp.12539.
Willems, J.L., Roelandt, J., De Geest, H., Kesteloot, H., Joossens, J.V., 1970. The left ventricular ejection time in elderly subjects. Circulation 42, 37–42.
Williams, S.W., Blunk, E.M., 2003. Sex differences in infant-mother attachment. Psychol. Rep. 92, 84–88. https://doi.org/10.2466/pr0.2003.92.1.84.
Yim, I.S., Quas, J.A., Rush, E.B., Granger, D.A., Skoluda, N., 2015. Experimental manipulation of the Trier Social Stress Test-Modified (TSST-M) to vary arousal across development. Psychoneuroendocrinology 57, 61–71. https://doi.org/10.1016/j.psyneuen.2015.03.021.
Zelenko, M., Kraemer, H., Huffman, L., Gschwendt, M., Pageler, N., Steiner, H., 2005. Heart rate correlates of attachment status in young mothers and their infants. J. Am. Acad. Child Adolesc. Psychiatry 44, 470–476. https://doi.org/10.1097/01.chi.0000157325.10232.b1.