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Child maltreatment under the skin: Basal activity and stress reactivity of the autonomic nervous system and attachment representations in maltreating parents

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CHAPTER 3

SALIVARY ALPHA-AMYLASE REACTIVITY TO INFANT CRYING IN MALTREATING MOTHERS

Reijman, S., Alink, L.R.A., Compier-de Block, L.H.C.G., Werner, C.D., Maras, A., Rijnberk, C., Van IJzendoorn, M. H., & Bakermans-Kranenburg, M.J. (2015). Salivary α -amylase reactivity to infant crying in maltreating mothers, *Child Psychiatry & Human Development*, 46, 589-599.

Abstract

Deviant physiological reactivity to infant stimuli has been suggested to underlie maladaptive parenting behavior. Our study involved 44 maltreating and 42 non-maltreating mothers. During a standardized cry paradigm, mothers listened to nine cry sounds of varying pitches. Saliva was collected at baseline, after each cry sound, and after a recovery episode. Salivary α -amylase (sAA) as a marker of autonomic nervous system (ANS) activity was assayed from saliva samples. Maltreating mothers showed lower overall sAA levels and an attenuated reactivity pattern to infant crying as compared to non-maltreating mothers. No effect of type of maltreatment (neglect only vs. neglect and abuse) was found. Furthermore, positive correlations between sAA and heart rate (HR) for nonmaltreating mothers differed significantly from non-significant correlations between sAA and HR for maltreating mothers. This suggests anomalous asynchrony between different aspects of the ANS in maltreating mothers. Results indicate a lack of functional autonomic (re)activity as a contributing risk factor to child maltreatment.

Keywords: Child maltreatment Salivary α -amylase Autonomic reactivity Infant crying

Introduction

The detrimental consequences for victims of child abuse and neglect are long-term and wide-ranging (Alink, Cicchetti, Kim, & Rogosch, 2012; Cicchetti & Toth, 2005; Spinhoven et al., 2010). The need for effective prevention and intervention programs urges extensive research on risk factors for child maltreatment, but the complex etiology of child maltreatment is challenging: Risk factors include various parent and child characteristics which dynamically interact with each other (Cicchetti & Valentino, 2006). Most studies have focused on (and found) parent characteristics as risk factors for child maltreatment, such as psychopathology and personal stress (Stith et al., 2009), and there is some evidence for different stress-reactivity to child signals in maltreating parents (McCanne & Hagstrom, 1996). However, most research has focused on physical maltreatment, or milder parenting problems, and severe neglect has often not been taken into account. This study aims to evaluate stress-reactivity to child signals in maltreating mothers who all had severely neglected their children, using salivary α -amylase (sAA) as a novel marker of physiological responsiveness.

A universally prevalent child behavior known to evoke differential responses from parents is infant crying (Soltis, 2004). Through crying infants communicate their distress and effectuate parental proximity and nurturance (LaGasse, Neal, & Lester, 2005), though some parents may perceive infant cries as aversive and respond harshly rather than provide the required care (Out, Pieper, Bakermans-Kranenburg, Zeskind, & Van IJzendoorn, 2010). Parents' harsh attempts to stop their infants from crying has been related to whether they judged the crying excessive, but not to the actual crying duration (Reijneveld, Van der Wal, Brugman, Hira Sing, & Verloove-Vanhorick, 2004). Neglectful parents may fail to respond at all (Crittenden, 1993).

Autonomic reactivity may be an underlying factor contributing to parents' differential responses to infant crying. Through the autonomic nervous system (ANS; part of the peripheral nervous system) the brain regulates the internal organs. The sympathetic and the parasympathetic systems (SNS and PNS, respectively) are the two main components that comprise the ANS (Larsen, Schneiderman, & DeCarlo, 1986). Stress typically activates the sympathetic division in order to procure bodily energy mobilization required for a behavioral response. Inversely, the parasympathetic system, which is involved in saving and recovering energy during rest, is inhibited during stress (Viamontes & Nemeroff, 2009). This results, for instance, in accelerated breathing, increased heart

rate (HR), and heightened skin conductance (SCL; electrodermal activity of the skin, a SNS measure). Additional to the comparison of normative and deviant reactivity patterns, differences in within-individual coherence of autonomic arousal may also point to anomalous disconnections between ANS indicators (El-Sheikh et al., 2009). For instance, correlations between HR and pre-ejection period (PEP; a measure of sympathetic cardiac control) were found to differ significantly in maltreating mothers versus non-maltreating mothers (Reijman et al., 2014), suggesting that in maltreating mothers, PEP may not serve its expected function of sympathetic cardiac boost.

A relatively recently discovered measure of ANS (re)activity is sAA. It is an important salivary enzyme whose main function involves the initiation of the digestion of macromolecules, such as carbohydrates and starch, in the oral cavity (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009). It is produced locally by the salivary glands, which are innervated by sympathetic and parasympathetic nerves stimulating its secretion into saliva (Nater & Rohleder, 2009; Baum, 1993). The focus on sAA as a biomarker of stress initiated when positive correlations were found between sAA and norepinephrine, a hormone of the sympathetic branch of the ANS (Chatterton, Vogelsson, Lu, Ellman, & Hudgens, 1996). Since then, studies have reported sAA response patterns similar to sympathetic response patterns during physical exercise and psychological stress (showing an increase in response to presentation of the stressor, and a recovery afterwards), as well as correlations between sAA and other sympathetic measures (Takai et al., 2004; El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; see Nater & Rohleder, 2009, for an overview). However, sAA has also been shown to be indicative of parasympathetic activity, as well as combined sympathetic and parasympathetic activity (e.g., Bosch, De Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003). This is congruent with the fact that the salivary glands are innervated by both sympathetic and parasympathetic nerves. The glands differ from one another in the extent of their sympathetic and parasympathetic innervation, as well as the amount of sAA they produce (Bosch, Veerman, De Geus, & Proctor, 2011). sAA can thus be used as a general measure of ANS (re)activity.

It has been suggested that infant crying leads to autonomic arousal in the caregiver, which consequently triggers caregiving behavior (Zeskind, Sale, Maio, Huntington, & Weiseman, 1985). Autonomic contagion (the sharing of autonomic arousal) in mother-child dyads has indeed been found (Ebisch et al., 2012). Maladaptive parenting responses might then

partly stem from deviant ANS arousal. In accordance with this principle, McCanne and Hagstrom (1996) concluded from a series of early studies that harsh or abusive caregiving responses appeared to be associated with autonomic *hyper*reactivity. However, as acknowledged in the review, inconsistency of findings among and within studies was notable. Recent studies have shown mixed findings as well. Congruent with the hyperreactivity hypothesis, increased SCL responsiveness to infant cry sounds predicted the use of harsh discipline during mother-child challenge tasks 9 months later (Joosen, Mesman, Bakermans-Kranenburg, & Van IJzendoorn, 2013b). However, HR *hypore*activity has also been related to less sensitive caregiving, while increased HR reactivity was observed in more sensitive mothers (Joosen et al., 2013a). This might be indicative of autonomic hypoarousal in relation to less adequate caregiving. Finally, mothers who showed autonomic hyperarousal during the Strange Situation (an experimental procedure to assess the quality of the attachment relationship between the child and the caregiver, in which they are separated and reunited in order to activate the child's attachment system through moderate stress) displayed more harsh/hostile caregiving during a dyadic free-play session, while maternal hypoarousal was related to observed disengaged parenting behavior (Sturge-Apple, Skibo, Rogosch, Ignjatovic, & Heinzelman, 2011). These findings could be suggestive of distinct ANS reactivity associated with child abuse and neglect: ANS hyperreactivity may be related to child abuse, while hyporeactivity may underlie neglect. However, the number of studies addressing severe neglect is small and abuse and neglect often co-occur (Euser et al., 2013), so underlying mechanisms may not be independent.

Studies on parenting, and especially child maltreatment, that include sAA as an autonomic measure are scarce. Congruent with the hyperreactivity hypothesis, increased sAA responsiveness to infant crying was associated with intended harsh caregiving responses in a sample of adult twin pairs, including parents and non-parents (Out, Bakermans-Kranenburg, Van Pelt, & Van IJzendoorn, 2012). Another study looked at sAA reactivity to a conflict discussion between family members, at least one of which (mother, father, or the adolescent child) had reported the occurrence of interparental physical aggression during the previous year (Gordis, Margolin, Spies, Susman, & Granger, 2010). In families with interparental physical aggression, fathers showed lower absolute sAA levels throughout the experiment, as well as attenuated sAA reactivity to the conflict discussion, and during recovery. Notably, in at least half of these families interparental physical aggression had

been witnessed by the adolescent child, which is considered a form of child emotional neglect (e.g., Barnett, Manly, & Cicchetti, 1993; Euser et al., 2013; Sedlak et al., 2010). There was no effect of interparental aggression on mothers' sAA levels. These studies, too, support the possibility of hyper- and hyporeactivity underlying harsh or abusive versus neglectful parenting, respectively.

An explanation for seemingly inconsistent findings may be that studies have focused on varied ranges of caregiving quality, used different stimuli, and included varying measures of ANS activity. This exposes the need for systematic substantiation and classification of child maltreatment, and standardized experimental tasks. Furthermore, there are several potentially confounding factors that should be taken into consideration in research on physiological arousal related to child maltreatment. For example, experiences of maltreatment in parents' own youth affects current dysfunctional parenting (Newcomb & Locke, 2001; Pears & Capaldi, 2001) as well as subsequent psychobiological development (e.g., Gordis, Feres, Oleski, Rabkin, & Trickett, 2010). Associated with both childhood maltreatment experiences (Spinhoven et al., 2010) and child maltreatment perpetration (Stith et al., 2009), depression has also been characterized by dysregulated (mainly increased) ANS (re)activity (see Carney, Freedland, & Veith, 2005, for an overview).

In the current study we aim to evaluate whether maltreating mothers differ from non-maltreating mothers in their autonomic reactivity to infant crying. ANS activity was measured with sAA, a non-invasive and relatively novel biomarker. Following methodological guidelines suggested by McCanne and Hagstrom (1996) we used a standardized cry paradigm to make results among studies more comparable. For the maltreating group, maltreatment was substantiated. In an explorative way, we distinguished between neglectful and additionally abusive mothers to evaluate whether differential reactivity was related to these subtypes of maltreatment. In accordance with previous studies, we tentatively expected hyporeactivity in neglectful mothers, and hyperreactivity for additionally abusive mothers, as compared to non-maltreating mothers. We considered mothers' own childhood maltreatment experiences and current depression tendencies to control for their possible effect on sAA reactivity to infant crying.

Method

Participants

We recruited 45 maltreating and 45 non-maltreating mothers at a mental health clinic. Mothers in the maltreating group received therapy that revolved around their parenting problems and were informed about the study by their therapists at the beginning of their treatment. We coded Child Protective Services (CPS) records to substantiate recent or ongoing maltreatment using the Maltreatment Classification System (MCS; Barnett et al., 1993). When records were inconclusive, we interviewed the mother's psychiatrist about her parenting problems using a semi-standardized interview. For three mothers, neither their records nor their psychiatrists provided proof of maternal maltreatment. In these cases, we conducted a Dutch adaptation of the Maternal Maltreatment Classification Interview (MMCI; Cicchetti, Toth, & Manly, 2003) with which absence of maltreatment was verified for two mothers. We considered them as non-maltreating in the analyses. One mother could not be reached for a follow-up MMCI and was excluded from analyses because of her inconclusive current maltreatment status.

Non-maltreating mothers were recruited in a clinical subdivision of the facility, where their child was in therapy for developmental or learning problems. To verify absence of maltreatment, the MMCI (Cicchetti et al., 2003) was used. For three mothers, incidents of sufficient severity to classify as maltreatment were coded from the interview. These mothers were counted as maltreating in the analyses. All currently maltreating mothers in our sample were in contact with CPS or were in therapy.

Three participants (3 %) had insufficient specimen volumes for sAA measures and were also excluded from analyses. The final sample consisted of 44 maltreating mothers and 42 non-maltreating mothers. Twenty-one maltreating mothers were neglectful only (henceforth abbreviated as NE) while 23 maltreating mothers were abusive *and* neglectful (AN).

Procedure

The study and its proceedings were approved by the Medical Ethics Review Committee for Mental Health Care (METiGG). All mothers gave informed consent for participation and, in the maltreating group, for researchers' access to the family files. As a compensation for participation, mothers received 40 Euros as well as travelling expenses.

Two individual appointments took place at the mental health facility. During the first session, mothers completed three computer tasks, of which the cry paradigm (Zeskind & Shingler, 1991) reported here was the first. sAA measures were collected during the task. Afterwards, mothers completed a questionnaire on health-related issues, such as smoking and exercising prior to the session, and on their family situation, including educational level, number of children, and children's ages. We administered the MMCI to the non-maltreating group. They took the Adult Self-Report (ASR; Achenback & Rescorla, 2003) home and were asked to bring it filled out to the second appointment. During the second session the Adult Attachment Interview (George, Kaplan, & Main, 1985) was conducted (data will be presented elsewhere). At the end of that visit mothers completed the short form of the Childhood Trauma Questionnaire (CTQ; Bernstein & Fink, 1998; Bernstein et al., 1994).

Measures

Maltreatment Classification System (MCS). We used the MCS (Barnett et al., 1993), a reliable and valid system to code incidents of maltreatment reported in the clinic's records from CPS and the child care office (Cicchetti, Rogosch, Gunnar, & Toth, 2010). Only incidents of *maternal* maltreatment were considered. According to the MCS, physical abuse is coded 'when a caregiver ... inflicts physical injury upon a child by other than accidental means', including hitting or kicking, violent handling (e.g., shoving, dragging), smothering, burning, shaking, or other, nondescript abuse. Physical neglect encompasses the failure to provide adequate food, clothing, shelter, health care, or hygiene, and a lack of adequate supervision that ensures the child's safety. Emotional maltreatment involves 'persistent or extreme thwarting of children's basic emotional needs' and 'parental acts that are harmful because they are insensitive to the child's developmental level'. We further distinguished between incidents of emotional maltreatment directed *at* the child (emotional *abuse*, e.g., belittling the child, or calling the child derogatory names) and incidents demonstrative of inattentiveness rather than rejection (emotional *neglect*, e.g., expecting an inappropriate level of responsibility from the child, exposing the child to interparental violence). In accordance with these operational definitions, we coded abuse (physical and/or emotional) and neglect (physical and/or emotional). No mother was found to have sexually abused any of her children. Coding was done by trained research assistants. Inter-rater reliability on 15 files was excellent, with $\kappa = .82$ for abuse and $\kappa = 1.00$ for

neglect. For the presence versus absence of maltreatment there was full agreement ($\kappa = 1.00$). Subsequently, all records were coded by two different research assistants and discrepancies were resolved through discussion. We found that all mothers in the maltreatment group had been neglectful towards their children, either physically or emotionally. 52 % ($n = 23$) of the maltreating mothers had also abused their child(ren) physically or emotionally.

Maternal Maltreatment Classification Interview (MMCI). The MMCI (Cicchetti et al., 2003) is a semi-structured interview that evaluates whether the mother has maltreated any of her children recently or during their lifetime. Mothers are asked about incidents of physical and emotional abuse and neglect, as well as sexual abuse, and about any contact the family may have had with CPS. We translated the interview into Dutch for this study. Coding was done by trained research assistants. We used the operationalized definitions of emotional and physical neglect and abuse from the MCS (Barnett et al., 1993; for details see above). Inter-rater reliability on 12 interviews was excellent, with full agreement for the presence versus absence of maltreatment ($\kappa = 1.00$). All interviews were coded by two research assistants and discrepancies were resolved through discussion.

Cry Paradigm. The cry paradigm was presented on a laptop with E-prime software. A 6-min baseline of neutral images was followed by three blocks with three cry sounds each (for details on the sounds' derivation, see Out et al., 2010). Within each of the blocks, sounds differed in fundamental frequency, congruent with the idea of infant crying as a graded signal of varying acoustic properties that communicate the cause's urgency (Gustafson, Wood, & Green, 2000). In each block, the three cries, of 500, 700, and 900 Hz, were presented in a random order. All sounds lasted 10 s, containing seven cry expirations, and were presented through Sennheiser HD202 headphones at a constant volume. The paradigm ended with another 4-min baseline of neutral images.

As a manipulation check, we tested whether the perception of the cry sounds differed for sounds with different fundamental frequencies. After each cry sound, mothers were presented with four questions on the extent to which they perceived the sound as urgent and aversive, the child as sick, and themselves as aroused (Out et al., 2012; Zeskind & Lester, 1978; Zeskind & Marshall, 1988). They answered on a 5-point scale, ranging from *very little* to *very much*. Each fundamental frequency was presented three times, resulting in 12 ratings per pitch. Following Out et al. (2010), who found one component underlying these twelve

ratings, averages were aggregated to form the overall perceived urgency. Cronbach's alphas ranged from .88 to .89. As expected, there was an overall main effect of pitch ($p < .001$), with a significant increase in perceived urgency from 500 to 700 Hz, from 500 to 900 Hz, and from 700 to 900 Hz ($ps < .001$). There was no main effect of current maltreatment status, nor an interaction effect of current maltreatment status with pitch ($ps > .54$).

To get acquainted with the cry sounds and the questions, participants practiced with one cry sound of 500 Hz. The cry paradigm has been used in previous studies with childless adults and parents and has been shown to evoke physiological stress responses, harsh caregiving intentions, and emotional irritation (Joosen et al., 2013b; Out et al., 2010; Riem, Bakermans-Kranenburg, Van IJzendoorn, Out, & Rombouts, 2012).

Salivary α -Amylase (sAA). After each pair of baseline images, each cry sound (save the practice trial), and at the end of the four recovery images, mothers were handed a salivette (Salimetrics Oral Swab). They were instructed to place it transversely under their tongue and keep it still for 90 s. E-prime was programmed to clock this interval, after which the experimenter sealed the salivette in a small tube. This led to a total of 13 sAA samples per participant. Samples were immediately stored frozen at $-20\text{ }^{\circ}\text{C}$ at the facility and transported regularly on dry ice to the university, where they were stored at $-80\text{ }^{\circ}\text{C}$.

After overnight thawing of the saliva at $4\text{ }^{\circ}\text{C}$, samples were centrifuged during 5 min at 2,773 g to remove debris, particles, and buccal cells. Saliva supernatants were then diluted 100-fold in physiological saline solution (Versylene1Fresenius Kabi, Zeist, the Netherlands; cat no. B230551) and mixed. Levels of sAA were measured in singlet, using a commercially available enzymatic colorimetric assay (cat no. 03183742; Roche Diagnostics, Mannheim, Germany). The amylase test kit consists of 4,6-ethylidene(G7)-1[4-nitrophenyl(G1)]-1,4-[α]-D-maltoheptaoside (EPS-G7) substrate and bacterial α -glucosidase ([α]-D-glucoside glucohydrolase; EC3.2.1.20). sAA degrades the EPS-G7 substrate to reaction products. α -glucosidase degrades uniformly all reaction products to 4-nitrophenol and glucose in the indicator reaction. The color intensity of the 4-nitrophenol formed in the indicator reaction is directly proportional to α -amylase activity. The test was applied on Integra 800 analyzer from Roche Diagnostics (Mannheim, Germany), according to the instructions of the manufacturer. CFAS (Calibrator For Automated Systems, cat no. 10759350) was used for calibration. The Roche routine amylase assay is standardized to the IFCC reference measurement procedure (Lorentz,

1999), guaranteeing worldwide comparability of the data. sAA activities were measured in IU/L at 37 °C and expressed in U/ml in the current report. Within-run imprecision for the control pool ranged from 0.7 to 2.7 % for the combined predilution step and the analysis across the entire study period. Between-run analytical imprecision was lower than 5 % throughout this study. Accuracy of the amylase activity procedure was guaranteed by regular participation in the Dutch External Quality Assessment Scheme (SKML, the Netherlands).

Outlying values on sAA measurements were found for six participants. Winsorizing is a commonly used procedure, also in studies on sAA (e.g., in Allwood, Handwerker, Kivlighan, Granger, & Stroud, 2011; De Veld, Riksen-Walraven, & De Weerth, 2012), to adjust outlying values without excluding them, thus doing more justice to the data of the complete sample. We used the winsorizing procedure following Tabachnik and Fidell (2001): Outliers were replaced with a value of .10 just above the highest *non*-outlying score (using standardized scores of -3.29 and 3.29 as cut-off). For the next more extreme outlier .10 was added to the preceding value, preserving the original order of scores. We also checked whether this procedure did not affect participants' original *individual* response patterns. When necessary, outlying values were adjusted taking into account the participant's original response pattern. Excluding the six participants with outlying values from the analyses did not affect the results, so we decided to report the results with these participants included. For 15 participants for whom at least 50 % of the values were present, missing values were imputed using curve fitting, by estimation of a quadratic curve. Next, mean sAA was calculated per *episode* (baseline; block 1, 2, 3 of cry sounds; recovery) in SPSS. Within the combined episodes, we found outliers for four more participants, which were winsorized following the same procedure. Finally, a square root transformation was applied to the combined episodes to correct for a positively skewed distribution. The transformed values showed no further outliers.

Childhood Trauma Questionnaire Short Form (CTQ-SF). The CTQ-short form (CTQ-SF; Bernstein & Fink, 1998; Bernstein et al., 1994; Thombs, Bernstein, Lobbstaël, & Arntz, 2009) is a self-report instrument that consists of 27 items (24 clinical items and 3 validity items) with which childhood experiences of abuse and neglect are retrospectively assessed. It has shown measurement invariance across four different populations (clinical and normal; Bernstein et al., 2003), and contains five subscales: physical abuse ($\alpha = .88$), emotional abuse ($\alpha = .89$), physical neglect ($\alpha = .70$), emotional neglect ($\alpha = .89$), and sexual abuse ($\alpha = .93$).

Each scale consists of five items with a 5-point scale ranging from *never true* to *very often true*. Two mothers (2 %) had missing data on the CTQ. These were imputed with the average of mothers with the same current maltreatment status.

Adult Self-Report (ASR). The ASR (Achenbach & Rescorla, 2003) is a measure of psychopathological symptoms in adults aged between 18 and 59 years. The total scale consists of several subscales, including anxious/depressed symptoms, aggressive behavior, and somatic complaints. We used a shortened version (77 items) of the ASR, and focused on the anxious/depressed subscale (18 items; $\alpha = .93$ in the current sample). Items were presented as statements referring to emotions, cognitions, and behaviors and participants rated how applicable the statement had been to them during the last 6 months, ranging from 0 (*not at all*) to 2 (*clearly or often*). For nine mothers (10 %) data on the anxiety/depression subscale were missing. These were imputed with the average of mothers with the same maltreatment status.

Heart Rate (HR), Respiratory Sinus Arrhythmia (RSA), Pre-ejection Period (PEP), and Skin Conductance Level (SCL). Throughout the cry paradigm electrocardiogram (ECG), impedance cardiogram (ICG), and SCL were measured using an ambulatory monitoring system (VU-AMS5 fs; TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). Average HR, RSA, PEP, and SCL were calculated per episode. HR, like sAA, is an autonomic measure that may reflect both underlying sympathetic and parasympathetic activity. PEP and SCL are markers of the SNS, while RSA reflects underlying PNS activity. Their correlation with sAA was examined to test for any disconnection within the autonomic system in maltreating mothers.

Data Analysis

We tested whether the four mothers excluded from analyses differed from the mothers included in our final sample ($N = 86$) in terms of ethnicity, educational level, child clinical diagnosis, childhood maltreatment experienced, anxious/depressed symptoms, maternal age and child age, and number of children. Next, we compared maltreating (NE and AN together as well as separately) and non-maltreating mothers on these variables. Additionally, we checked whether maltreating and non-maltreating mothers differed on hearing problems, exercise, smoking, and substance (ab)use. We examined the associations between sAA levels and all background variables. Controlling for maternal age (which correlated with sAA levels during blocks 1 and 3; see below), we computed partial correlation coefficients to see whether correlations

between sAA and other autonomic measures differed significantly for maltreating and non-maltreating mothers. Correlations between cardiovascular/electrodermal measures and sAA within corresponding episodes (e.g., HR baseline with sAA baseline) were examined, as well as between cardiovascular/electrodermal levels of each episode with the *following* sAA episode (e.g., HR baseline with sAA block 1), because sAA responses are slower than those of the cardiovascular/electrodermal measures.

As for the primary hypothesis, to evaluate the association between current maltreatment status and autonomic reactivity to cry sounds, we performed repeated measures ANCOVAs. Current maltreatment status (maltreating vs. non-maltreating) was entered as between-subjects factor. The five *episodes* (baseline, block 1, 2, 3, and recovery) were entered as within-subjects factor. None of the background variables on which maltreating and non-maltreating mothers differed were related to baseline levels of sAA. However, since they might moderate the sAA *response pattern*, they were initially entered as covariates in the repeated measures ANCOVAs. Number of days mothers had been in therapy before participation (with a 0 assigned to non-maltreating mothers), and the time of the first sAA measurement were also entered as covariates. Leaving the non-significant covariates out, childhood maltreatment experiences, anxious/depressed symptoms, maternal age, smoking, and number of days in therapy were included as covariates in the final analyses. With a more exploratory aim, we repeated the analyses with three levels of current maltreatment status (NE, AN, and non-maltreating) as between-subjects factor.

Results

Preliminary Results

Excluded mothers ($n = 4$) were less anxious/depressed than mothers included in the sample ($N = 86$), on average had more children, and fewer excluded than included mothers were of Caucasian ethnicity ($ps < .02$). Excluded and included mothers were similar on childhood maltreatment experiences (total CTQ scale), educational level, maternal age, children's mean age, and whether children had a clinical diagnosis ($ps > .27$). Table 1 displays characteristics of maltreating mothers and non-maltreating mothers. Maltreating mothers (both NE and AN) had more childhood maltreatment experiences (total CTQ scale) than non-maltreating mothers [$t(72.98) = -2.85, p = .01$] and were more anxious/depressed, $t(76.80) = -2.90, p = .01$. Somewhat more maltreating

mothers (both NE and AN) had a lower educational level, $\chi^2(3, N = 84) = 7.90, p = .048$. Mothers and their children in the maltreating group were significantly younger than their counterparts in the non-maltreating group, $t(78.28) = 4.58, p < .001$ and $t(84) = 4.31, p < .001$, respectively. There were no differences between NE and AN mothers ($ps > .39$). Since maternal age and children's average age were highly correlated ($r = .80$), only maternal age was included as a potential covariate in further analyses. Maternal age was not correlated with sAA baseline ($p = .18$). The two groups did not differ on ethnicity, number of children or whether their children had a clinical diagnosis ($ps > .16$).

Furthermore, fewer maltreating mothers than non-maltreating mothers had exercised in the week prior to the research appointment, $\chi^2(1, N = 84) = 8.60, p = .003$; specifically, fewer NE mothers had exercised compared to non-maltreating mothers, $\chi^2(1, N = 61) = 9.44, p = .002$. More maltreating mothers than non-maltreating mothers had smoked on the morning of the research appointment, $\chi^2(1, N = 84) = 8.77, p = .003$; specifically more AN mothers than non-maltreating mothers had smoked, $\chi^2(1, N = 64) = 10.27, p = .001$. The two groups were similar on hearing problems and substance (ab)use ($ps \geq .10$). None of the variables on which maltreating and non-maltreating mothers differed (i.e., educational level, maternal age, CTQ sum score, ASR depression scores, exercise, and smoking) were related to sAA baseline levels ($ps > .14$). Maternal age correlated positively with sAA during blocks 1 and 3 ($ps < .05$).

Correlations Between Autonomic Measures

Partial correlations between HR baseline and sAA block 1, HR block 1 and sAA block 1, HR block 1 and sAA block 2, and HR block 2 and sAA block 2 differed significantly for maltreating and non-maltreating mothers ($ps < .05$; Table 2). For non-maltreating mothers, all correlations between HR and sAA were positive, and those between HR baseline and sAA block 1, as well as HR block 1 and sAA block 1 were significant ($p < .05$). For maltreating mothers, the correlations between HR and sAA were non-significant in the negative direction. For RSA, PEP, or SCL correlations with sAA did not differ between maltreating and non-maltreating mothers ($ps > .05$).

Differences in sAA for Maltreating and Nonmaltreating Mothers

There was a main effect of current maltreatment status, $F(1, 79) = 8.13, p = .01$, partial $\eta^2 = .09$, with maltreating mothers showing lower sAA levels than non-maltreating mothers (Fig. 1). Furthermore, we found an

overall (multivariate) interaction effect of current maltreatment status with episode, $F(4, 76) = 3.39, p = .01$, partial $\eta^2 = .15$. Contrast analyses showed that maltreating mothers' stable sAA levels differed from non-maltreating mothers' sAA increase from baseline to block 1, $F(1, 79) = 5.63, p = .02$, partial $\eta^2 = .06$. Furthermore, maltreating mothers' stable sAA levels differed from non-maltreating mothers' decreasing sAA from block 1 to recovery, block 2 to recovery, and block 3 to recovery [$F(1, 79) = 11.49, p = .001$, partial $\eta^2 = .13$; $F(1, 79) = 5.23, p = .03$, partial $\eta^2 = .06$; $F(1, 79) = 4.45, p = .04$, partial $\eta^2 = .05$, respectively; see Fig. 1].

Differences in sAA Between NE, AN and Nonmaltreating Mothers

We found a main effect of current maltreatment status, $F(2, 78) = 4.30, p = .02$, partial $\eta^2 = .10$, with NE and AN mothers showing overall lower sAA levels than non-maltreating mothers. There were no differences between NE and AN mothers in overall sAA levels or reactivity patterns ($ps > .14$).

Table 1
Sample Characteristics for Maltreating, Neglectful, Abusive, and Non-maltreating Mothers

	Maltreating (total group) <i>N</i> = 44	Neglectful Only <i>n</i> = 21	Abusive and Neglectful <i>n</i> = 23	Non-maltreating <i>N</i> = 42
<i>Ethnicity (%)</i>				
Caucasian	93	91	96	93
<i>Completed education (%)</i>				
None	2.3	5.0	0.0	0.0
Elementary/short track secondary	44.2 ^a	45.0	43.5	22.0 ^b
Secondary/vocational	44.2	40.0	47.8	51.2
College/university	9.3 ^a	10.0	8.7	26.8 ^b
	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
Childhood malt exp	48.28 (21.25) ^a	48.88 (21.08) ^a	47.74 (21.86) ^a	37.40 (13.39) ^b
Anxiety/depression	12.85 (8.36) ^a	12.10 (8.62) ^a	13.54 (8.24) ^a	8.37 (5.80) ^b
Maternal age	37.84 (7.35) ^a	38.86 (6.78) ^a	36.91 (7.87) ^a	44.14 (5.30) ^b
Children's age	9.34 (4.97) ^a	10.00 (5.34) ^a	8.74 (4.65) ^a	13.53 (3.97) ^b
Number of children	2.48 (1.25)	2.38 (1.24)	2.57 (1.27)	2.40 (1.11)

Note: Childhood malt exp childhood maltreatment experiences.

^a differed significantly from ^b on the respective variable at $p < .05$.

Table 2

Differences in sAA – HR Partial Correlations Between Maltreating and Non-maltreating Mothers

	sAA Baseline		sAA Block 1		sAA Block 2		sAA Block 3		sAA Recovery	
	M	N-m	M	N-m	M	N-m	M	N-m	M	N-m
HR										
Baseline	-.10	.33	-.04 ^a	.41* ^b	-	-	-	-	-	-
Block 1	-	-	-.08 ^a	.40* ^b	-.20 ^a	.29 ^b	-	-	-	-
Block 2	-	-	-	-	-.19 ^a	.31 ^b	-.11	.31	-	-
Block 3	-	-	-	-	-	-	-.12	.28	-.04	.16
Recovery	-	-	-	-	-	-	-	-	.03	.10

Note: M maltreating, N-m non-maltreating, sAA salivary α -amylase, HR heart rate. For maltreating group $n = 41$, for non-maltreating group $n = 36$; controlled for maternal age.

^{a b} correlations differed significantly between maltreating and non-maltreating mothers, $p < .05$.

* $p < .05$

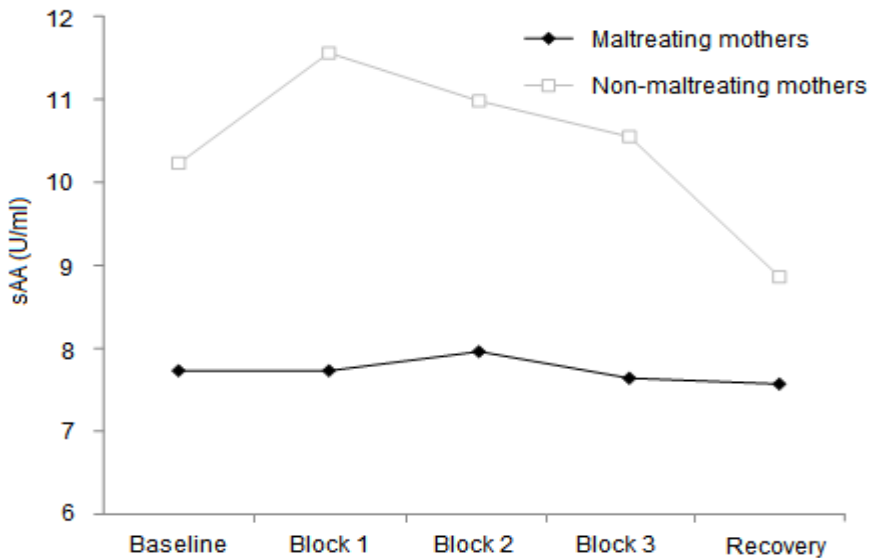


Figure 1. Overall lower sAA levels for maltreating than for non-maltreating mothers; baseline to block 1: no increase in sAA levels for maltreating mothers; from block 1, block 2, block 3 to recovery: stable sAA for maltreating mothers ($N = 86$: maltreating mothers, $n = 44$; non-maltreating mothers, $n = 42$).

Discussion

We found that maltreating mothers had overall lower sAA levels than non-maltreating mothers. Though traditionally studies have focused mainly on sAA reactivity, sAA levels have been shown to be stable across time and conditions, their variation largely explained by genetic factors (Out, Bakermans-Kranenburg, Granger, Cobbaert, & Van IJzendoorn, 2011). They are therefore a valuable indicator of autonomic activity. Furthermore, while listening to infant crying maltreating mothers showed attenuated sAA arousal as compared to non-maltreating mothers. The difference in autonomic reactivity patterns was evident despite the fact that perceived urgency of the sounds was equal for both groups. The lack of autonomic arousal in response to children's distressed arousal might reflect limited empathy in maltreating mothers (Ebisch et al., 2012). Since empathy implies an understanding of the child's needs and in that way facilitates an appropriate response, maltreating mothers might experience a disconnection from their children on a physiological, affective, and behavioral level, impeding adequate responsiveness even to common caregiving demands. This is congruent with a study by Sturge-Apple et al. (2011), in which ANS

hyporeactivity was found to be associated with maternal disengagement from her child.

Somewhat contrary to our expectations, there were no differences in overall sAA levels or reactivity patterns between NE and AN mothers. This might be because of insufficient statistical power due to small subgroup sizes: Whereas the power to find a medium-sized difference between the maltreating and the non-maltreating mothers amounted to .86 (taking into account the covariates), the power to find a significant effect for the difference between the NE and AN mothers was only .70, and lower for smaller effects. Furthermore, in our sample, all mothers neglected their children, so that a true distinction between perpetrated abuse and neglect was impossible. Alternatively, autonomic hypo(re)activity for both abusive and neglectful parents might reflect a common behavioral disconnection from their children. Both abusive and neglectful mothers showed significantly less affection, play behavior, and initiated less interaction with their children than comparison mothers during systematic home observations (Bousha & Twentyman, 1984). In an early review of the literature, Wolfe (1985) argued the possibility that on an everyday level, abusive parents do not necessarily respond more punitively or harshly to children's negative signals than other parents, but do fail to provide constructive and consistent discipline strategies as well as positive affect. The cry paradigm we used presents mothers with a mild child-related stressor of an intensity that mothers are likely to encounter on a daily basis. It is conceivable that in quotidian contexts, such as that of short infant cry bouts, abusive mothers too show a lack of engagement with their child, rather than instant hyperirritability.

Evaluating whether different patterns of dysfunctional autonomic reactivity to more extreme stressors are associated with different types of child maltreatment remains useful. Future studies would continue to benefit from an operationalized distinction between emotional and physical abuse and neglect. A challenge in this regard is that different types of maltreatment often co-occur, as they did in our sample (Euser et al., 2013; Manly, Cicchetti, & Barnett, 1994). Large sample sizes are therefore needed to link differential risk factors to subtypes of maltreatment.

Our findings appear to be in contrast with McCanne and Hagstrom's (1996) conclusion that more studies provided evidence for a relation between physiological *hyper*reactivity and abusive parenting. However, they pointed out that results were inconsistent among studies, which is possibly due to methodological differences such as the characteristics of

experimental stimuli. For that reason, we used a standardized cry paradigm that has been proven effective in other studies (Joosen et al., 2013a, 2013b; Out et al., 2010; Riem et al., 2012) and makes results among studies more easily comparable. Using the same cry paradigm, autonomic hyperreactivity was found to predict maternal harsh disciplining 9 months later (Joosen et al., 2013b), but harsh discipline might be reflective of maternal over-involvement rather than disengagement. It may therefore be fundamentally different from maltreatment in our sample, of which severe child neglect was a dominant trait. This demonstrates that results are difficult to compare when different operationalizations of parenting are used. Our results support the basic notion that ANS arousal is a requirement for sensitive parenting, for which promptness of response is a main condition (Joosen et al., 2013a). Congruently, in a sample of mothers where normative, hyper-, and hypoarousal patterns of the ANS were distinguished during the Strange Situation, mothers showing autonomic hypoarousal scored highest on observed insensitive parenting behavior (Sturge-Apple et al., 2011). There may be an optimal level of arousal that promotes sensitive parenting, but based on the current state of the research in this area, no definite conclusions can be drawn yet.

Furthermore, we found that significant positive correlations between HR and sAA for non-maltreating mothers differed significantly from non-significant correlations between HR and sAA for maltreating mothers. Asymmetry *between* the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenomedullary (SAM) system has been identified as a correlate of maltreatment experiences and aggression in adolescents (Gordis, Granger, Susman, & Trickett, 2006; Gordis, Granger, Susman, & Trickett, 2008). The importance of studying the systems' interaction has already been emphasized (Bauer, Quas, & Boyce, 2002). Although it has been recognized that measures *within* the ANS generally do not correlate well (Bosch et al., 2011; Folkow, 2000), it is the pattern of significant differences between correlations in our sample that is striking and suggests that future study of within-system dissociations might be worthwhile as well.

We turn to some of the methodological aspects of this study. The disadvantage of presenting standardized cry sounds in an experimental setting is not knowing to what extent the results can be generalized to mothers' natural caregiving context. However, mothers were tested in an environment familiar to them. Standardized paradigms also make results more easily comparable among studies.

A second limitation is that the maltreating and non-maltreating groups differed on several aspects: maltreating mothers and their children were younger, had a somewhat lower educational level, had smoked more (despite our request not to do so) and exercised less previous to the research appointment. This profile in itself might be telling in terms of the stressors maltreating mothers face and the control they have over their lives. It is consistent with the findings that parental low educational level is a risk factor for child maltreatment (Euser et al., 2013) and low parental age is (modestly) associated with risk for abusive and neglect (Stith et al., 2009). Regarding the effect of these variables on autonomic (re)activity, we tested their potential confounding effect. Smoking and maternal age were significant as a covariate on certain contrasts in the analyses. By including smoking and maternal age as covariates (among others) we precluded their confounding influence on the results. Finally, we did not measure salivary flow rate. sAA levels are affected by saliva flow which may depend on methodological factors such as collection material and sampling duration (Bosch et al., 2011). However, saliva collection was standardized in the current study (participants kept the swab under their tongue for 90 s), in which case sAA activity (U/ml) and sAA output (U/min) are highly correlated.

In conclusion, maltreating mothers showed lower sAA levels and an attenuated sAA reactivity pattern in response to infant crying. This might reflect maltreating mothers' disengagement from mildly stressful child signals, such as they encounter on a daily basis. Autonomic hypo(re)activity may partly underlie their impaired ability to adequately respond to their children on a behavioral level. It has been suggested that children who show physiological hyper- or hyporeactivity may require different types of intervention, and low physiological responsiveness in particular might be associated with low responsiveness to intervention (for a review see Gunnar & Fisher, 2006). Similarly, our findings provide a broader understanding of the biological and experiential profile of maltreating mothers and might therefore be useful for the development of prevention and intervention programs from which they can optimally profit.

Summary

We wanted to evaluate whether maltreating mothers differ from non-maltreating mothers in their autonomic reactivity to infant crying. We tentatively expected autonomic hyperreactivity in abusive mothers, and hyporeactivity in mothers who neglected their children. 44 maltreating

and 42 non-maltreating mothers participated in the study. Maltreatment was substantiated by coding the families' records from CPS and the child care office. All mothers were found to have neglected their children, while 52 % was additionally abusive. During a standardized cry paradigm, mothers listened to nine infant cry sounds that varied in pitch. Saliva was collected at baseline, after each cry sound, and after a recovery episode. sAA was assayed from saliva samples and used as a measure of ANS activity. Maltreating mothers showed lower overall sAA levels and a flat reactivity pattern to infant crying as compared to non-maltreating mothers. We found no effect of type of maltreatment (neglect only vs neglect and abuse), which may be explained by the fact that all mothers in the sample were neglectful, impeding a clear distinction between maltreatment subtypes. Furthermore, positive correlations between sAA and HR for non-maltreating mothers differed significantly from non-significant correlations between sAA and HR for maltreating mothers. This may indicate anomalous asynchrony between different components of the ANS in maltreating mothers. Results are interpreted in light of the functional sharing of autonomic arousal between mother and child, which might be reflective of maternal empathy for her child's distress. The lack of functional autonomic (re)activity can thus be seen as a risk factor contributing to child maltreatment.