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Does Touch Matter? The Impact of Stroking versus Non-Stroking Maternal Touch on Cardio-

Respiratory Processes in Mothers and Infants.

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Abstract

The beneficial effects of touch in development were already observed in different types of skin-to-skin care. In the current study, we aimed at studying potential underlying mechanisms of these effects in terms of parasympatho-inhibitory regulation. We examined the specific impact of affective maternal stroking versus non-stroking touch on the cardio-respiration of both mothers and infants in terms of respiratory sinus arrhythmia (RSA). We compared a 3-min TOUCH PERIOD (stroking or non-stroking touch) with a baseline before (PRE-TOUCH) and after (POST-TOUCH) in 45 dyads (24 stroking/21 non-stroking touch) with infants aged 4-16 weeks. We registered mother-infant ECG, respiration and made video-recordings. We calculated RR-interval (RRI), respiration rate (fR) and (respiratory corrected) RSA and analyzed stroking mean velocity rate (MVR) of the mothers. ANOVA-tests showed a significant different impact on infants' respiratory corrected RSA of stroking touch (increase) versus non-stroking touch (decrease). Further, during and after stroking touch, RRI significantly increased whereas fR significantly decreased. Non-stroking touch had no significant impact on infants' RRI and fR. In the mothers, RRI significantly decreased and fR significantly increased during the TOUCH PERIOD. The mothers' MVR occurred within the range of 1-10 cm/s matching with the optimal afferent stimulation range of a particular class of cutaneous unmyelinated, low-threshold mechano-sensitive nerves, named c-tactile (CT) afferents. We suggest CT afferents to be the a potential missing link between the processing of affective touch and the development of physiological and emotional self-regulation. The results are discussed with regard to the potential role of CT afferents within the building of early self-regulation as part of a multisensory intuitive parenting system and the importance to respect this ecological context of an infant in research and clinical applications.

Keywords: Maternal affective touch; Stroking and non-stroking touch; C-tactile afferents; Respiratory Sinus Arrhythmia; Cardio-respiratory processes; Mother-infant Interaction.

Does Touch Matter? The Impact of Maternal Touch on Cardio-Respiratory Processes in

Mothers and Infants.

The bonding phase between mothers and infants is a period during which the basis of an infant's primary social relationship is established. By means of a multimodal set of reciprocal exchanges, they both contribute to the organization of rhythmic cycles of mutual regulation that promote the building of the infant's further social development (Feldman, 2007). Papoušek (2007) labeled these cycles of preverbal communication as 'the realm of non-consciously regulated intuitive behavior and implicit relational knowledge' (2007, p. 258) pointing to the intuitive or biologically predisposed nature of it. Within these cycles of intuitive parenting, touch occupies a major position, evoking physiological, epigenetic and neuroendocrine exchanges that are known to be important for the development of a well-functioning stress regulation system (e.g., Feldman, Rosenthal, & Eidelman, 2014; Meaney, 2001; Sharp et al., 2012; Uvnäs-Moberg, 1996; Walker & McGlone, 2013). Indeed, from a series of animal studies, it has been shown that maternal factors such as tactile stimulation in the form of nurturing licking and grooming are critical in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, and vice versa that maternal separation impairs long term HPA-responsiveness (e.g., Champagne et al., 2008; Franklin, Linder, Russig, Thöny, & Mansuy, 2011; Levine, 2001; Uchida et al., 2010). These studies (Champagne et al., 2008; Franklin et al., 2011; Levine, 2001; Meaney, 2001; Uchida et al., 2010) showed a clear link between the deficiency of early touch experiences and a later disadvantaged brain/mind development as a function of the interplay between the reactivity to stress of the HPA axis and the myelination maturation of axons of HPA-related brain regions. One of the reasons for the beneficial effect of maternal proximity can be found on the level of the glucocorticoid receptor gene expression in the hippocampus and prefrontal brain regions (e.g., Curley & Champagne, 2016). Optimal nurturing and tactile stimulation boosts this gene expression which enhances optimal feedback

regulation of the HPA axis and the reset of cortisol secretion after being exposed to a stressor (for a review, see Walker & McGlone, 2013). Since the functioning of the HPA axis is integrated within the entire psychophysiological regulation system, the impact can be found on physiological, emotional as well as cognitive self-regulation (Friedman, 2007; Thayer & Lane, 2000, 2009).

Processes of self-regulation are mediated by connections between subcortical and cortical brain regions as well as between the central and autonomic nervous system by direct and indirect pathways via the nervus vagus (i.e., cranial nerve X) and the sinoatrial node of the heart (Benarroch, 1993; Thayer & Lane, 2000, 2009). Because of these interconnections, the activity of the heart, and in particular its connection with the respiration cycle, offers an excellent window into autonomic activity, i.e., the sympatho-excitatory and parasympatho-inhibitory output of a stress-response (Berntson, Cacioppo, & Quigley, 1991; Thayer & Lane, 2000, 2009). It has been accepted that both branches of the autonomic nervous system are simultaneously at work and that sympathetic activity can be associated with heart rate (HR) acceleration during inspiration and parasympathetic activity with HR deceleration during expiration (Berntson et al., 1997; Grossman, Vanbeek, & Wientjes, 1990). Therefore, the heart rate variability (HRV), i.e., the alternation between increased and decreased HR in relation with the respiratory cycle can be considered as an index for autonomic activity (Berntson et al., 1997).

Within HRV, respiratory sinus arrhythmia (RSA) reflects the specific component related with the parasympatho-inhibitory impact on the heart, mediated by the nervus vagus and related to processes of (psycho)physiological development or self-regulation (Berntson et al., 1997). RSA is obtained by calculating the mean difference between the longest heart period associated with expiration and the shortest heart period associated with inspiration for each respiratory cycle (i.e., peak-valley method, Grossman et al., 1990). The measure of RSA is interesting in

infant research since it is a non-invasive manner to obtain insight into the maturation of their regulatory system (Van Puyvelde et al., 2015). Indeed, RSA maturation in infants is not fully developed in the first months of life (Bar-Heim et al., 2008) and touch may thus play a role to fulfill this developmental task.

One can differentiate two types of touch provided during maternal care to infants, i.e., stroking touch and non-stroking touch. The beneficial effect of non-stroking touch on the infant's cardio-respiratory system has been shown in several studies on early skin-to-skin contact in full-term newborns immediately after birth (e.g., Bystrova et al., 2003; Christensson et al., 1992; Winberg, 2005) and in preterm infants during skin-to-skin kangaroo care (e.g., Bergman, Linley, & Fawcus, 2004; Bloch-Salisbury, Zuzarte, Indic, Bednarek, & Paydarfar, 2014; Boju, Krishna, Uppala, Chodavarapu, & Chodavarapu, 2011; Feldman & Eidelman, 2003; Ibe et al., 2004). Moreover, in a study of Van Puyvelde et al. (2015), it was shown that such processes of cardiorespiratory transfer are present during the first two months of life but weaken towards three months of life. In this study, infants adjusted their RSA-levels to those of their mothers when lying on their breast; an ability that was mediated by the cardiac rhythm and that (Van Puyvelde et al., 2015). Another caregiver's cardiac transfer was shown in a study with preterm infants (Bloch-Salisbury, Zuzarte, Indic, Bednarek, & Paydarfar, 2014) in which the infant's respiratory control was influenced by the caregiver's cardiac rhythm during skin-to-skin contact. Since maternal-infant physiological transfer in terms of cardiac adjustments have been reported even prenatally (Van Leeuwen et al., 2009), an early postnatal physiological transfer during close body-contact may be the expression of a continuation from intra-uterine needs, preserved postnatally to reassure optimal regulation during early bonding (Van Puyvelde et al., 2015).

Stroking touch adds an extra mechanical stimulation factor provided by the stroking frequency of the caregiver. It has been proposed that a population of gentle touch sensitive

unmyelinated nerves, called c-tactile (CT) afferents, play a fundamental role in the beneficial impact of stroking touch (McGlone, Vallbo, Olausson, Löken, & Wessberg, 2007; McGlone, Wessberg, & Olausson, 2014; Vallbo, Olausson, & Wessberg, 1999). CT afferents are a group of mechanosensitive fibers residing in the hairy skin areas of the body and the face. They are absent in the glabrous skin areas such as the hand palms and soles of the feet. These unmyelinated, low mechanical threshold nerves respond optimally to gentle low force touch with a medium stroking velocity rate between 1 and 10 cm/s (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009; Mcglone et al., 2007) at a neutral typical skin temperature (Ackerley et al., 2014).

In adults, gentle stroking at this velocity is reported to be perceived as pleasant (Essick et al., 2008) and to evoke brain activation in the posterior insular cortex, mid-anterior orbitofrontal cortex (e.g., Gordon et al., 2013; McGlone et al., 2012; Olausson et al., 2002), the anterior cingulate cortex and amygdala (e.g., Gordon et al., 2013). All of these CT afferent sensitive brain regions also receive inputs from thermoreceptors and visceral afferents that are functional in the psychophysiological regulation of an individual (Craig, 2002). This is not surprising, knowing that they are recognized as the key-regions in the neurovisceral integration model (Thayer & Lane, 2009). This model explains how the inhibitory communication between prefrontal regions and the amygdala, and subsequently between the amygdala and the autonomic medullary output, is responsible for the regulatory modulation of HRV parameters such as RSA (Thayer & Lane, 2009) and may therefore justify why CTs could form a potential missing link between touch and the development of physiological and emotional self-regulation. Moreover, recent research shows that the same network of both limbic brain regions (e.g., the insular cortex) and parasympatho-inhibitory activity is stimulated during soft brushing touch. Soft brushing evoked activation in the insular cortex of infants of 4 weeks (Tuulari et al., 2017) and 8 weeks (Jönsson et al., 2018) of evoked decreased HR and increased attentional

engagement (Fairhurst et al., 2014). Van Puyvelde et al. (2015) reported that occasional non-intended maternal stroking touch resulted in an immediate phasic RSA-response in the infant.

Obviously, other factors than the above ascribed cardiorespiratory transfer during non-stroking touch and CT afferent activation during stroking touch may be responsible for observed regulatory effects in infants. Mothers will intuitively comfort their infants by means of a multimodal package that combines a variety of regulation aspects. For instance, in a former study of Korner and Thoman (1972), it has been shown that the underlier of maternal soothing behavior is not the tactile but rather the vestibular component of a behavior. That is, in a series of different soothing behaviors, the combined effect of being lifted and maintained in an upright position overruled any potential effect of tactile contact (Korner & Thoman, 1972). Nevertheless, in a study of Birns et al. (1966) no significant differences were found between diverse soothing techniques. The most important clarifications provided by Korner and Thoman (1972) was the length of stimulation period [i.e., a short stimulation to evoke an orienting response versus a continuous stimulation in Birns et al. (1966)] as well the fact that the different soothing techniques in Birns et al. (1966) were applied in an upright position which was designated to be a defining factor in Korner and Thoman (1972).

Hence, it is not surprising that the social context in which touch occurs and the multimodality inherent to the ecological context of mother-infant nurturing care are defining in the successfulness of the impacted psychophysiological integration which implies that this ecological context should be respected in an experimental design. For instance, the infant brain responses to affective stroking found in Jönsson et al. (2017) could not be replicated in Pirazzoli, Lloyd-Fox, Braukmann, Johnson and Gliga (2018), which the authors ascribed to their strict laboratory setting that lacked the natural multimodal mother-related stimulation versus the presence of mother arms in Jönsson et al. (2017). Indeed, when touch is a necessary condition to realize the normal development of a social brain (Walker & McGlone, 2013), it

would be expectable that a successful stimulation occurs within a caring interactive context of intuitive parenting (Papoušek, 2007) as well. The latter is supported by the findings of Croy et al. (2016) that mothers stroke their infants intuitively at a velocity rate matching the above mentioned optimal CT afferent stimulation.

In the current study, we examined the relationship between intuitively delivered maternal non-stroking versus stroking touch and RSA-reactivity in both infants and their mothers. We used an experimental pre-during-post design, whilst monitoring maternal-infant ECG and respiration and recording video of the mothers' stroking activity to examine the MVR. In order to safehold optimal ecological conditions, the data were collected in the mother's home. Additional to Fairhurst et al. (2014) who limited physiological measuring to pulse-oximetry in 10 s time windows, we measured ECG and respiration—only the combination of both offers reliable insight into parasympathetic activity (Grossman et al., 1990). RSA comprises, on top of parasympathetic activity, respiratory and somato-motor metabolic parameters (Beauchaine, 2001) that need be taken into account for final interpretations with regard to the engagement of the vagal system. These aspects remain unknown when analyses are based on HR only (Grossman et al., 1990). Moreover, the need to measure respiration is even more demanding in an infant population (Ritz et al., 2012; Van Puyvelde et al., 2014, 2015) because of the immature respiratory control (e.g., Giddens & Kitney, 1985). Also, a trade-off needs to be made between a duration of signal epoch long enough to compute the analysis and short enough to minimize non-stationarity, which brings an epoch for parasympathetic measures usually to an average of 3 min (minimal 1 min) (Berntson et al., 1997). Therefore we have chosen time windows that followed these minimal requirements for psychophysiological research. Finally, the mothers were asked to remain the infant in a similar position through the entire experiment (baseline and experimental touch period) in order to avoid a potential confounding orienting response at the level of the vestibular system.

Methods

Participants

The study was approved by the local ethics committee of the University Hospital XXX (XXX 143201629352). We recruited 45 mothers from prenatal classes and a private midwife's office; 24 mothers participated in the stroking touch condition, 21 mothers were assigned to the small non-stroking touch condition. The mothers who agreed to participate were contacted a few weeks before the agreed upon meeting moment. The average age of the mothers was 30.92 (*SD* = 3.35; range 26-42 years) and the average age of the infants was 9.32 weeks (*SD* = 2.63; range 4-14 weeks). All of the infants (24 males, 21 females) were healthy full-term born babies with an Apgar score above seven. The mean birth length was 49.1 cm (*SD* = 2.46; range 42-55 cm) and the mean birth weight was 3.22 kg (*SD* = 0.44; range 2.5-4.68 kg). Four dyads were excluded from the control group. Two because of an unreliable respiratory signal in the infant (due to fussiness) and two others because the baby was too fussy to finish the experiment. Hence, the final analyses were conducted on 41 dyads, 24 in the stroking touch group and 17 in the non-stroking touch group.

Apparatus

For the ECG and the respiration registration, the BioRadio TM system (Great Lakes NeuroTechnologies Inc., Cleveland, OH, USA) was used which allows synchronized mother-infant registration of ECG and respiration signals. The BioRadio consisted of a Primary Module, which is a wireless acquisition system that allows free movement. It is a non-invasive system that already has been used in other studies in the context of mothers and infants (e.g., Van Puyvelde et al., 2014, 2015). Video recordings were made with synchronized Sony Handycams type HDR-CX160 and HDR- SR11E. A Statistical Package for Social Sciences Version 25.0 (SPSS 25.0) was used to perform the statistical analyses.

Monitoring of the Physiological Signals

Two standard single-channel ECG registrations (II derivation) were used (i.e., one for the mother, one for the infant) in correspondence with standard configurations, i.e., one electrode on the upper right side and the lower left side of the chest. Two grounding electrodes were placed, one on the back of the mother and one on the back of the infant. The ECG signals were recorded with a 960-Hz sampling frequency. For the ECG we used a lowpass Bessel filter order 4 with a lower cutoff of 100 Hz, and for breathing we used lowpass Bessel filter order 2 with a lower cutoff at 1 Hz. To register the breathing movements, the mother wore a thoraco-abdominal respiratory effort belt, and the infant wore a pediatric belt.

Questionnaires

In the full design, the Touch Experiences and Attitudes Questionnaire (TEAQ) (Trotter, McGlone, Reniers, & Deakin, 2018) and the Postnatal Depression Scale (EDPS) were used. The TEAQ inquires upon the subject's attitudes with regard to affective touch along with their experiences regarding affective touch. The questionnaire comprises the following components: Current Social Touch (CST), Current Intimate Touch (CIT), Childhood Touch (ChT), Attitude to Personal Grooming (APG), Attitude to Intimate Touch (AIT) and the Attitude to Unfamiliar Touch (AUT). The EDPS is an instrument to examine the risk for postnatal depression (Cox, Holden & Sagovsky, 1987). In the scope of the current study, we will not report the results of the questionnaires and only focus on physiological events.

Procedure

The data were collected during home visits. Based on the recommendations for best practice healthy infant skin care (Blume-Peytavi et al., 2016), mothers were asked to control the room temperature by thermostat on 22-24° Celsius. After the installation of the mobile lab, the mothers were asked to fully undress their infant except from the diaper. Subsequently, the

electrodes and respiration belts were fit and the mothers were asked to choose a seated resting position with their infant that felt comfortable to stroke the infant. To avoid an impact of a cardiac transfer from the mother to the infant as demonstrated in Bloch-Salisbury et al. (2014) and Van Puyvelde et al. (2015), we asked the mothers to not hold the infant on their chest. There was a stroking touch and non-stroking touch group. For both groups, the experiment consisted of three within-subjects conditions, i.e., a pre-touch baseline condition (PRE-TOUCH, 3 min), a stroking or non-stroking touch period during which the stroking mothers were asked to stroke their infant and the non-stroking mothers were asked to maintain skin-contact by holding the infant's hand or foot (stroking/non-stroking TOUCH, 3 min 20 s) and a post-touch baseline condition (POST-TOUCH, 3 min) (see Figure 1). In the non-stroking group, the mothers were allowed to say a small word now and then to the infant when he or she was seeking contact in order to confirm their presence for the infant. We preferred this manner of togetherness above a sustained non-touch and silent situation during the overall period of the three conditions to avoid a still-face or at least a sustained non-interactive atmosphere. Only so, we could reassure that a potential negative or zero-result in the control condition would be the result of the lack of non-stroking touch and not of a negatively experienced interaction. Through the article, 'TOUCH PERIOD' will refer to the 3 min 20 s period during which mothers touched their infant, be it by stroking touch or non-stroking touch.
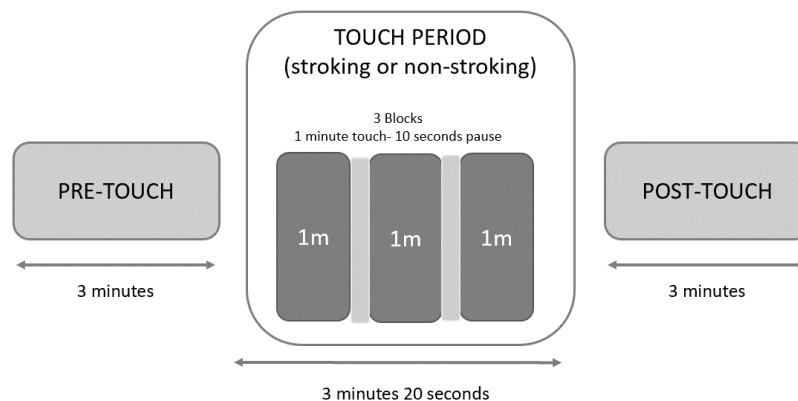
Figure 1. Design with three conditions, i.e., PRE-TOUCH, TOUCH PERIOD, POST-TOUCH. PRE-TOUCH and POST-TOUCH were baseline blocks during which the mothers were asked to not interact with the infant. The TOUCH PERIOD refers to a period of stroking touch in the experimental group and non-stroking touch in the control group. During stroking touch, the mothers were instructed to stroke their infant as they would normally do. In the control group, the mothers were asked just to touch their infant without stroking. With the 1 min stroking - 10s pause alternation during TOUCH we intended to avoid exhaustion of the CT afferent reactivity.

During both PRE-TOUCH and POST-TOUCH, the mother was asked to not interact with the infant, unless they thought the infant was experiencing discomfort. During the TOUCH PERIOD in the stroking touch group, in correspondence with a study of Croy et al. (2016), the mothers were instructed to stroke their infant as they would normally do, in a straight line and on a body location that felt natural to them. To avoid exhaustion of the CT afferent reactivity, we imposed an alternating scheme of 1 min stroking and 10s pause (see Figure 1). The mothers were informed by the experimenter when to proceed to a next condition or when to stroke/touch and when to pause. When the mother indicated that she and the infant felt comfortable to start the experiment, the PRE-TOUCH condition commenced. After the experiment, the electrodes were removed, the infant was immediately dressed again and the questionnaires were completed by the mother.

Physiological Signal Analysis

The ECG and respiration data were visually inspected for artifacts and (in)correct detections. In the case of ectopic beats or erroneous detections, the data were manually corrected (removal of erroneous detection/artifact followed by a cubic spline interpolation; corrections <1%). Other undesired events in the physiology due to crying, caresses during baseline conditions, rocking the infant were removed as artefacts based on a frame by frame inspection of the video recordings. The timing of the detected R-wave was used to generate the

RR interval (RRI). For each testing block, maternal and infant respiration frequency (fR), RRI, and RSA were calculated. RSA was computed using the peak-valley method to reflect vagal tone (Grossman et al., 1990), as advised for infant research (Ritz et al., 2012; Van Puyvelde et al., 2015). In the time-domain peak-valley method the mean difference between the longest heart period associated with expiration and the shortest heart period associated with inspiration for each respiratory cycle is calculated (Grossman et al., 1990). We used VivoSense software version 3.1 (Vivonoetics, San Diego, USA) to analyze the recorded signals. The programmed algorithms of the VivoSense software correspond with the advised standards of Grossman et al. (1990) and Grossman, Karemaker, and Wieling (1991). For infant settings, we adjusted the ECG RR-lockout period for R-wave picking to 0.1. The breath detector algorithm sets a minimum peak-trough value that must be exceeded for a minimum-maximum pair to be denoted as an actual breath. This helps to eliminate non-respiratory small visceral movements from being marked as breaths. This minimum value is denoted as the minimum tidal volume (MTV) in the properties of the breath channel. The default value for the MTV was adjusted to infants to 15–30 ml. In agreement with Grossman et al. (1990), the inspiratory and expiratory windows were moved forward 750 ms to accommodate the phase shifts occurring between heart period and respiration (e.g., Eckberg, 2003). Additionally, the VivoSense R-wave detection and the RSA calculation account for violations of the Nyquist criterion. The Nyquist-criterion is the requirement that the sampling rate is at least twice as high as the frequency of interest. As stated in the introduction, this requirement is easily violated in infant research because of their immature respiratory control. That is, when the respiration rate is too irregular and/or rapid to allow the detection of two succeeding RRI-periods during one inspiration or expiration, the Nyquist-criterion is violated (Ritz et al., 2012; Rother, Witte, Zwiener, Eiselt, & Fischer, 1989; Van Puyvelde et al., 2014, 2015). VivoSense accounts for violations of the Nyquist-criterion and scores the breaths with no detectable peak-valley RSA as zero.

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Data Preparation: Respiratory Controlled RSA (RSA*corr*)

To disentangle effects of *f*R on RSA, a statistical within-subject regression approach is accepted (e.g., Grossman & Taylor, 2007). Consistent with this approach, we conducted a series of within-subject regressions on the averages of the data of each experimental block, predicting infants' RSA from its *f*R. Residuals from these regressions were collected and used to control for the effect of fR on RSA (Grossman et al., 1991; Grossman & Taylor, 2007). An additional common issue in psychophysiological research is the large between-individual variation in RRI, RSA and *f*R measures. Hence, within-subjects z-transformations were applied, as recommended in a previously published methodology (e.g., Bush, Hess, & Wolford, 1993; Van Puyvelde et al., 2014, 2015).

Video-analysis

Mean Velocity Rate (MVR). To analyze the mothers' MVR, we made use of the movement tracking software, Tracker 4.9.8, from the open source Physics by Douglas Brown (2018). Prior to analysis, the video recordings of the experimental stroking periods were cut into three blocks of one minute. Each block was separately analyzed which allowed to control consistency in velocity rate. After calibration, the auto-tracking feature was used in case a point mass (i.e., a central recognition point, in our case the sticker on the finger of the mother) was recognized through the entire length of the stroking period. If the point mass was not recognized through the entire stroking period, the manual tracking function was used (i.e., a frame by frame analysis). The velocity rate is the calculated distance divided by time. The final stroking velocity rate used for analysis was the MVR across the entire TOUCH condition (Croy et al., 2016).

Infant motor behavior. The video recordings were checked on motor activity and/or fussiness of the infant since this has an impact on RSA of both adults (e.g., Grossman et al.,

2004) and infants (Bazhenova, Plonskaia,& Porges, 2001; Ritz et al., 2012; Van Puyvelde et al., 2012). Based on the coding system of Bazhenova et al. (2001), the infants were categorized in a quiet motor behavioral state for > 95% of the time. To meet this standard, two fussy infants were excluded for further analysis (see section Participants). We did not perform this analysis on the mother group since they were instructed to stroke (see also limitations of the study in the Discussion).

Statistical Analysis

Presentation raw data. We presented an overview of the raw data with exploratory one-way repeated measures analysis of variance (ANOVA) with condition (PRE-TOUCH, stroking/non-stroking TOUCH, POST-TOUCH) as within subjects variables and RSA, RRI and fR as dependent variables are given.

Main analysis. The main analysis consisted of three 3 x 2 (condition [PRE-TOUCH, stroking/non-stroking TOUCH, POST-TOUCH] x group [stroking, non-stroking) mixed ANOVAs with condition as within-subjects, group as between subject factor and respiratory corrected RSA, RRI and fR as dependent variable to evaluate the difference in impact of stroking versus non-stroking touch on infants' RSA, RRI and fR and subsequently on mothers' RSA, RRI and fR.

Gender and age effects. We tested for gender and age effects, by a series of 3 x 2 (condition [PRE-TOUCH, stroking/non-stroking TOUCH, POST-TOUCH] x gender [boys, girls]) mixed ANOVAs and a 3 x 3 (condition [PRE-TOUCH, stroking/non-stroking TOUCH, POST-TOUCH] x age [< 8 weeks, 8-12 weeks , >12 weeks]) mixed ANOVA with condition as within-subjects factor, age as between subject factor and RSA, RRI and fR as dependent variables. The cutoffs of the age groups were based on the transition periods in physiological

adjustments in infants observed in Van Puyvelde et al. (2015). We also tested maturation effects in the infants by a Kendall's tau correlation test.

MVR. Furthermore, we conducted an independent-samples t-test to compare the velocity rate with the mean velocity rate (MVR) observed in Croy et al. (2016). Finally, a Pearson correlation test was conducted to check consistency of the mothers' touch through the three parts in the touch period.

In all of the repeated measure ANOVA tests, an extra evaluation of the proportion of variance (i.e., effect size or partial eta-squared, ηp^2) was made along with pairwise comparisons by post hoc tests with the critical p-value for significance adjusted with Bonferroni correction. Further, the illustration and report confidence intervals was based on the within-subjects approach for repeated measures of Cousineau (2005) that normalizes confidence intervals for within-subject designs.

Questionnaires. A Kendall's Tau correlation test was conducted to examine potential links between the EPDS and the TEAQ. Further, we examined correlations between the questionnaires and the physiological baselines of both mothers and infants and the physiological reactivity in the stroked infants. For the latter we used delta Δ scores of TOUCH and POST-TOUCH versus PRE-TOUCH for RSA*corr*.

<div align="center">

Results

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Overview of Respiratory Uncorrected Raw Physiological Data

Table 1 displays an overview of the One-way ANOVA (PRE-TOUCH, TOUCH and POST-TOUCH) tests on respiratory uncorrected RSA, RRI and fR in the mother and infant group. All of the data were inspected for outliers based on the z-standardizations (z = +/- 2.58). In the infant group two infants were excluded for the RSA analyses having z-values > 2.56 and one infant was excluded for the RRI analyses having a z-value > 2.56.

Main Analysis

Table 1
Means (SD) and analyses using one-way repeated measures ANOVA of raw RSA values (in ms), raw RRI values (in s) and raw fR values(respiration rate per minute) during PRE-TOUCH, TOUCH and POST-TOUCH of the mother group and infant group.

		PRE-TOUCH M (SD)	TOUCH M (SD)	POST-TOUCH M (SD)	df	F	p	ηp²	Bonferroni
Mothers Stroking Group	RSA raw	61.447 (9.132)	54.937 (9.034)	62.110 (8.996)	2, 42	2.811	.072	.118	/
	fR	18.57 (1.024)	19.441 (1.380)	18.234 (0.982)	2, 44	4.595	**.015***	.173	Post <Touch, p = .042
	RRI	0.861 (0.020)	0.834 (0.027)	0.864 (0.023)	2, 44	7.650	**<.001***	.258	Pre >Touch, p = .015 Post >Touch, p = .014
Mothers Non-stroking Group	RSA raw	48.594 (9.919)	39.27 (9.204)	41.656 (6.029)	2, 26	2.993	.068	.187	/
	fR	16.76 (1.556)	20.015 (1.375)	18.809 (1.624)	2, 24	10.151	**.001***	.458	Pre < Touch, p < .001
	RRI	0.800 (0.020)	0.782 (0.023)	0.805 (0.029)	2, 30	2.636	.088	.149	/
Infants Stroking Group	RSA raw	8.879 (1.310)	10.503 (1.728)	11.575 (3.308)	2, 44	12.598	**< .001***	.364	Pre <Touch, p = .027 Pre < post, p < .001
	fR	43.797 (3.506)	41.330 (2.454)	39.564 (7.488)	2, 46	7.418	**.002***	.244	Pre > Post, p = .010
	RRI	0.427 (0.014)	0.423 (0.011)	0.438 (0.014)	2, 44	5.538	**.007***	.201	Touch < Post, p = .006
Infants Non-stroking Group	RSA raw	9.203 (1.320)	9.214 (1.239)	8.011 (1.677)	2, 30	2.514	.098	.144	/
	fR	42.390 (2.847)	42.494 (3.017)	43.008 (3.736)	2, 32	0.118	.889	.007	/
	RRI	0.388 (0.011)	0.388 (0.007)	0.384 (0.013)	2, 32	0.619	.545	.037	/

Note. Signifcant *p*-values are indicated in boldface with an asterisk (*).

There was a significant difference between the impact of stroking versus non-stroking touch on the infants' RSA*corr* [$F(2, 74) = 8.524$, $p < .001$, ηp² = .187], on RRI [$F(2, 76) = 4.686$, $p = .012$, ηp² = .110] and on fR [$F(2, 78) = 3.896$, $p = .024$, ηp² = .91]. Repeated contrasts showed a significant difference between the response in RSA*corr* in the stroked (i.e., increase) versus non-stroked infants (i.e., decrease) from the touch period to the POST-TOUCH, $p = .032$ and a tendency to significance from PRE-TOUCH to the touch period, $p = .082$, (see Figure 2). The lack of increase and presence of decrease in the non-stroking group was not due to ceiling effects. Firstly, the visual difference in starting RSA*corr* between both groups during PRE-

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TOUCH was not significant, $t(37) = 0.558$, $p = .580$, and secondly, in case of a ceiling effect, the stroked infants could not had increased to a level significant higher than the start-point of the non-stroked infants, $t(37) = -2.495$, $p = .017$.

With regard to RRI, the repeated contrasts showed that the increase in RRI in stroked infants versus the decrease in RRI in non-stroked infants was significantly different after the touch period, $p = .003$. Also here, these results cannot be due to ceiling effects given that the group with highest RRI increased and the group with smallest RRI decreased (see Table 1 and Figure 2). Finally, although the general ANOVA showed a significant impact of stroking on the fR, the repeated contrasts indicated no significance during the touch period ($p = .103$) and after the touch period ($p = .185$). In the mother group, there was no significant impact on RSA$corr$ [$F(2, 68) = 0.035$, $p = .965$, $\eta p^2 = .001$] and no interaction effect with the group (stroking vs non-stroking) [$F(2, 68) = 0.592$, $p = .592$, $\eta p^2 = .015$]. There was a significant impact on RRI [$F(2, 74) = 9.071$, $p < .001$, $\eta p^2 = .197$] and no interaction effect with group [$F(2, 74) = 0.248$, $p = .781$, $\eta p^2 = .007$]. RRI decreased from the PRE-TOUCH ($M = 0.843$; $SD = .041$) to the TOUCH condition ($M = 0.820$; $SD = .041$), $p = .002$ Bonferroni corrected, and increased again to the POST-TOUCH condition ($M = 0.847$; $SD = .044$), $p = .004$, Bonferroni corrected. There was a significant interaction effect in fR $F(2, 68) = 6.243$, $p = < .003$, $\eta p^2 = .155$]. Repeated contrasts showed that the difference between PRE-TOUCH and TOUCH was larger in the non-stroking mothers than in the stroking mothers, $p = .006$ (see also Table 1).

Infants
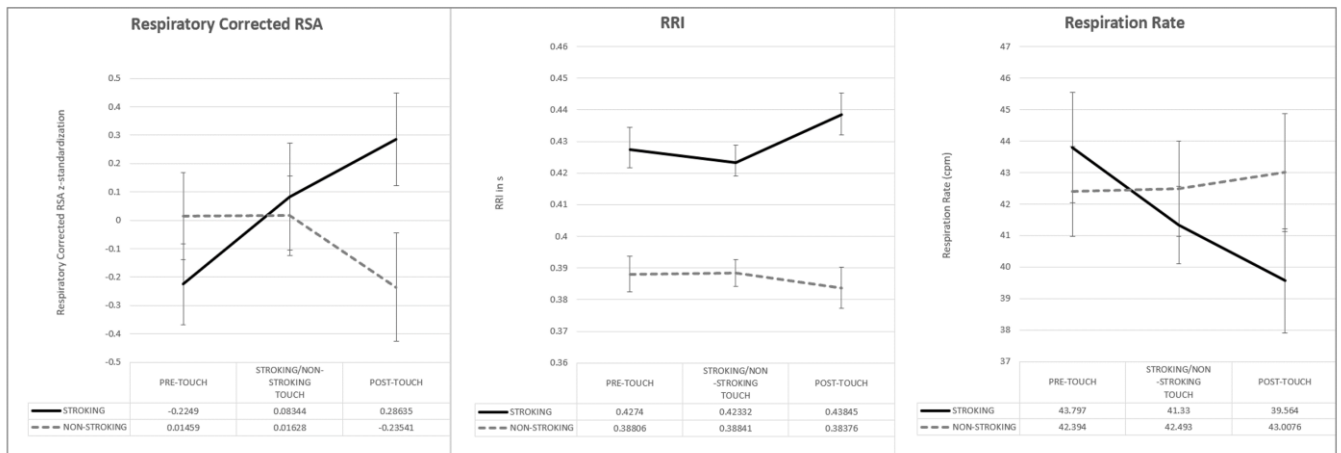**stroking versus non-stroking group**

Figure 2. Infants' respiratory corrected RSA, RRI and *f*R in the stroking versus non-stroking group. There was a significant difference between both groups in all of the variables. Both RSA and RRI increased during and after stroking touch and decreased after non-stroking touch. The respiration rate decreased during and after stroking touch and showed no changes in non-stroked infants.

Gender and Age Effects

Gender and age effect patterns. We found no gender or age effects on RSA*corr,* RRI or *f*R, nor within the infant group, neither in the mother group (range *p*-values .214 to .387). Because of the rather small powers that were observed in the the age and/or gender effect tests (i.e., within a range of .093 to .543), we studied the descriptive graphs as well. There were no salient peculiarities except one (non-significant) gender pattern (with however a significant contrast test, $p = .049$) within the non-stroking touch infants, $F(2, 28) = 2.505$, $p = .100$, $\eta p^2 = .152$, observed power = .460. This test showed that the boys and girls responded differently to non-stroking touch (there was no difference with regard to stroking touch). The boys showed decreased parasympathetic regulation after the non-stroking maternal touch withdrawal whereas the girls did not , $p = .049$ (see Figure 3). With a larger power, a significance could be hypothesized.
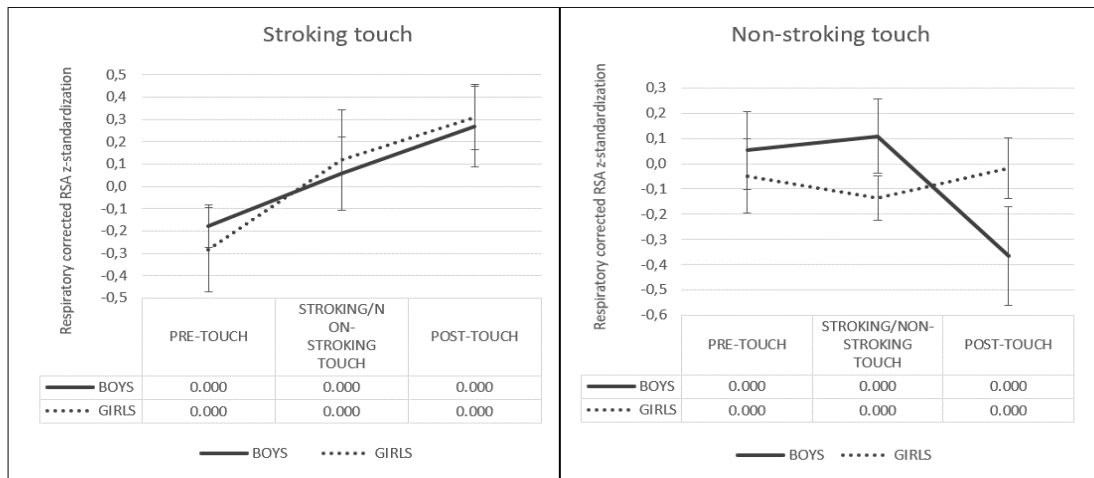
Gender patterns in the infant group

Figure 3. Illustration of the (non-significant) gender pattern with significant contrast in the non-stroking touch group on infants' RSA*corr*. Boys and girls responded similarly to stroking touch whereas only boys lost parasympathetic regulation after non-stroking touch withdrawal of the mother.

Maturation effects. We found no maturation effects, $r = .064$, $p = .585$ for RSA, $r = .021$, $p = .859$ for RRI, $r = .141$, $p = .230$ for fR.

MVR

The MVR was calculated for 21 mothers. Three video recordings were not of sufficient quality to conduct a velocity rate analysis. The mean velocity rate provided by the mothers was 7.28 cm per s ($SD = 3.2227$; range $2.35 – 15.11$; CI [5.81;8.74] cm/s). There was no significant difference between the MVR in the current study ($M = 7.28$ cm/s) and the MVR in Croy et al. (2016) ($M = 8.4$cm/s) found in Croy et al. (2016), $t(20) = -1.598$, p $= .126$. A Pearson correlation test showed that MVR remained consistent over the three minutes, there was a positive correlation between MVR in minute 1 and 2 ($r = .923$, $p < .001$), between MVR in minute 1 and 3 ($r = .841$, $p < .001$) and between MVR in minute 2 and 3 ($r = .865$, $p < .001$).

Questionnaires

The responses on the EPDS showed that none of the participating women scored above the clinical border (i.e., > 12) for postnatal depression (*M* = 5.77; *SD* = 3.1). Nevertheless, we found a significant negative correlation between EPDS-scores and the subscale of 'Attitude to Personal Grooming' (APG) at the TEAQ which inquires to the experienced pleasure of taking care of the body and using body lotions, bath soap, etc. (τ = .-360, *p* = .034). Within the stroking touch group, there was a positive correlation between both the TEAQ Total Score and Attitude to intimate touch AIT-subscale with the reactivity in infants' RSA*corr* from PRE-TOUCH to POST-TOUCH period (τ = .290, *p* = .047 for Total score; τ = .348, *p* = .019 for AIT).

Discussion

In the current study, we investigated the impact of maternal nurturing stroking versus non-stroking touch on both infants' and mothers' RSA, *f*R and RRI. In the non-stroking touch group, during the TOUCH PERIOD, mothers were instructed to sit together with their infant and just hold his or her feet or hand. The main analysis showed a significantly different response to stroking touch than to non-stroking touch. Stroked infants showed increased RSA*corr* and RRI during and after stroking whereas these variables did not change during and even decreased after the touch period in the non-stroked infants. The *f*R decreased during and after touch in the stroked infants whereas it maintained unchanged in the non-stroked infants. This combination of increased RSA*corr*, increased RRI and decreased *f*R in the stroked infants points to parasympatho-inhibitory activity, partly driven by heart rate and partly driven by respiratory factors.

During the first two months of age, infants are capable to passively adjust their RSA to their mother's when lying in close body contact (Van Puyvelde et al. , 2015). The observed effect in the current study could not be the consequence of a similar cardiorespiratory transfer since we asked the mothers to not hold the infant on the chest in the heart zone. Moreover, unlike the passive RSA adjustments in Van Puyvelde et al. (2015), the RSA reactivity to

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stroking touch in the current study was not age-dependent. Our infant population comprised infants younger and older than 2 months of age and no age effects were found. Therefore, we suggest that there are diverse developmental stages to benefit from various types of RSA stimulation. Maternal-infant care intuitively changes in relation with the infant's age from nurturing close body contact during the first weeks of life towards an always more varied and socially embedded pattern of interaction (Papoušek, 2007) that however needs the foundation of regulation build during the first weeks of life.

The observed difference in impact of stroking versus non-stroking touch on infants' RSA showed that stroking was an important factor within the observed regulation. In agreement with Croy et al. (2016), the mothers, intuitively, stroked their infant at a MVR that matched the optimal CT afferent stimulation range of 1-10 cm/s. Hence, we suggest that the intuitively supplied maternal gentle stroking stimulated the CT afferents and with that the parasympathetic activity observed during and after stroking in the infants. Since Korner and Thoman (1972) suggested that the vestibular component of a behavior is stronger than the tactile component, we attempted to control for this. Nevertheless, the relationship between CT afferents and the vestibular system should be closely examined in further research. Both systems are mechanical sensitive and it would not be surprising that a similar population of nerves to CT afferents might exist in the labyrinths of the inner ear that respond to gentle rocking motions.

With regard to the maturation of the CT afferent system, it has been shown that CT afferent like reactivity is already detectable from 4 weeks of age onwards (Tuulari et al., 2017; Jönsson et al., 2018) which may suggest its developmental importance. Several studies have shown that gentle stroking induces the release of oxytocin in animals (e.g., Okabe, Yoshida, Takayanagi, & Onaka, 2015; Uvnas-moberg, Bruzelius, Alster, & Lundeberg, 1993;Winslow, Noble, Lyons, Sterk, & Insel, 2003), human adults (Carmichael et al., 1987; Light, Grewen, & Amico, 2005; Turner, Altemus, Enos, Cooper, & McGuiness, 1999) and infants (Matthiesen,

Ransjo-Arvidson, Nissen, E., & Uvnäs-Moberg, K., 2001). Moreover, touch has been shown to regulate sympathetic HPA-reactivity in rats (e.g., Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000), blood pressure in adults (e.g., Light et al., 2005; Lund, Lundeberg, Kurosawa, & Uvnäs-Moberg, 1999) and HR in infants (Fairhurst et al., 2014) and adults (Light et al., 2005). Walker and McGlone (2013) suggested CT afferents to be a mediator between oxytocin and social bonding since both systems have the same endogenous effects whereas Devries, Glasper and Detillion (2003) suggested oxytocin at its turn to be a mediating factor between social support and HPA-axis suppression. Both perspectives are complementary and logical, knowing that oxytocin receptors are widely spread throughout the brain, sharing limbic brain locations with serotonin and dopamine pathways (Walker & McGlone, 2013). These cooperating systems can be considered as a "neuronal twin" in terms of healthy social behavior. Indeed, in a study of Fries et al. (2005), children that were exposed to parental neglect during the first 12 months of life did not respond with similar elevated oxytocin to physical contact than control subjects, even when already adopted for 2-3 in a securely caregiving home situation. The first 12 months of life is the period during which it is suggested that infants need to build RSA and their inhibitory parasympathetic regulation (Bar-Heim et al., 2008). The above mentioned recent research of Tuulari et al. (2017) and Jönsson et al. (2018) showed that, although myelinization is not yet fully developed in young infants, the unmyelinated transition system is already functional at 8 weeks of age; CT afferent optimal stroking activated—similar to that in adults—the insular cortex and temporal lobe (Jönsson et al., 2018). Therefore, we suggest that the first 12 months of life may be a critical developmental window for both the integration of the CT afferent system and a healthy stress regulation system and that early touch is a necessary but not sufficient condition for this.

The significant decrease in RRI and increase in ƒR, observed in the mothers during TOUCH, was probably due to the combination of the physical activity and affective

engagement during the experiment (as observed in Van Puyvelde et al., 2014). Both factors are known to impact cardio-respiration and need be taken into account regarding the interpretation of physiological findings (e.g., Beauchaine, 2001; Ritz et al., 2012; Van Puyvelde et al., 2014, 2015). However, the increase in fR was larger in the non-stroking mothers than the stroking mothers. However, afterwards, some mothers reported that they were sometimes worried whether the infant would stay still during the experiment without any stimulation which could explain this result. Their concern was also supported by the fact that we had to exclude four infants from the control group. Nevertheless, to increase insight, in further studies, a vanilla baseline (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992) should be used, during which mothers, for instance, would already been engaged in a stroking and mentally engaged activity.

The gender analyses were indicative of a tendency for boys to be more sensitive to the withdrawal of maternal non-stroking touch than girls. Although these results need to be interpreted with greatest cautiousness due to a rather small observed power, it might be interesting to further examine gender-related reactivity patterns in infants with regard to both and RSA maturation in general.

With regard to the questionnaires, although all the mothers scored within the non-clinical window for postnatal depression, we found a significant negative correlation between the attitude to personal grooming (APG) and the EPDS-scores. This correlation could point to a risk factor that is often overseen in screening instruments for postnatal depression which is the amount of self-care that a mother foresees for herself (e.g., Jack, 1991; Knudson-Martin & Silverstein, 2009). A high-scoring APG-subscale on the TEAQ could be seen as a particular protective factor in the difficult daily challenge of a mother to not efface herself. It would therefore be valuable to further examine the usefulness to include self-care related aspects in postnatal depression screening instruments and preventive care. The correlations found between the TEAQ and the infants' RSA*corr* reactivity scores could be indicative of certain

intergenerational patterns of touch comfort but should be further studied in larger populations to come to reliable conclusions.

The present study comprised both merits and limitations. First of all, we want to underline the importance of including respiratory measures in order to analyze RSA-variables in a reliable manner because of the natural irregularity of young infants' respiration cycles and to make clear interpretations with regard to underlying autonomic activity. Secondly, the experiment was conducted within the ecological context of the infant. A first limitation of the study was that we should had provided a vanilla baseline for the mothers that took into account the impact of physical and mental engagement (Jennings et al., 1992). Further, aspects such as age and gender effects and touch attitudes should be further examined in larger populations.

In summary, we found that during and after maternal stroking touch, the parasympatho-inhibitory regulation of infants increases, which was not the case during with regard to non-stroking touch. Intuitively, mothers stroked their infants at a CT afferent optimal velocity. It would be interesting to further examine whether there is a critical period of CT afferent stimulation in the building of psychophysiological integrity, whether long-term touch programs may be beneficial for the parasympatho-inhibitory maturation of infants and whether there is an intergenerational transferred pattern of touch attitude and reactivity.

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