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Infants' stress responses and protest behaviors at childcare entry and the role of care providers

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Abstract

During the transition from home to childcare, 70 15-month-old infants were videotaped, and their negative emotions were rated. Infants' attachments to mothers were assessed prior to child care entry and to care providers five months later using the Strange Situation Procedure (SSP). Infant heart rate was monitored at home, during adaptation to childcare (mothers present), and during subsequent separations. Respiratory sinus arrhythmia (RSA) was computed from the beat-to-beat measures of heart rate to reflect vagal tone, which is reduced during chronic states of stress, and was collected upon *Arrival*, during in-group *Play*, and when in the *Group* more generally. All infants responded to childcare entry with low RSA levels indicating stress. However, during adaptation with the mother present, RSA was higher for securely attached infants. On the first separation day, 35.3% of the infants fussed and cried extensively. These intense protests predicted later secure attachments to care providers, which adaptively helped to reduce stress, especially in infants who protested extensively, as if summoning their mothers back. Because extensive protest suggests limited regulatory capacities, infants risk overburdening the stress system when left unsupported.

KEYWORDS
care provider–child attachment, center-based care, child temperament, stress regulation, vagal tone

1 | INTRODUCTION

Nonparental childcare has become an increasingly important and potentially beneficial support for parents and young children (Lamb & Ahnert, 2006; NICHD Early Childcare Network, 2002; NICHD Early Childcare Network & Duncan, 2003). The early onset of childcare, however, has complex psycho-biological and behavioral effects on children's functioning. Full day placement in childcare is physiologically stressful for most infants and toddlers (e.g., Geoffroy et al., 2006; Vermeer & van IJzendoorn, 2006), and the transition from home to group care can be especially stressful. Although the presence of the mother during an initial period of adaptation might buffer stress responses if a secure infant–mother attachment has been established (e.g., Ahnert et al., 2004; Rauh et al., 2000), some infants display heightened levels of negative emotion, immobilization, and despair in that context. Reactions seemed to vary depending on whether infants have enacted regulatory capacities that allow them to modulate stress effectively as indicated by an easy temperament (Fein, 1995; Fein et al., 1993). It is, however, still unclear whether and to what extent certain actions at entry can minimize stress and facilitate children's adaptation

to the new care environment. In this paper, we examine children's cardiac responses throughout the transition from home to childcare.

1.1 | The interface between children's cardiac responses, negative emotions, and temperament

Cardiac responses are regulated by the combined contribution of both branches (i.e., sympathetic and parasympathetic) of the autonomic nervous system. Environmental challenges stimulate the sympathetic nervous system, but also depress the calming of the parasympathetic nervous system via vagal influences on the heart. As a result, heart rate and measures of the cardiac vagal tone indexed by the spontaneous pattern of beat-to-beat heart rate known as respiratory sinus arrhythmia (RSA) have proven to be reliable indices of stress (e.g., Lewis et al., 2012; Porges, 1995). Specifically, lower cardiac vagal tone results in faster heart rate and lower RSA amplitude and is associated with higher stress levels. Children with difficult temperaments respond with higher behavioral intensity and tend to have higher RSA whereas more socially reticent children have lower RSA (Kagan & Fox, 2006). Moreover, as detailed in the polyvagal theory (Porges, 2011), the neural regulation of RSA via the vagus is neuroanatomically and neurophysiologically linked to areas in the brainstem that control facial expression and vocal intonation to form an integrated social engagement system as behavioral counterparts of cardiac stress responses (e.g., Porges, 2011; Porges & Furman, 2011). Thus, consistent with this linkage between vagal regulation of the heart and the social engagement structures involved in emotions, the present study focuses on children's RSA, temperament, and negative emotions related to childcare entry. Whereas the HPA axis (which has been exclusively explored at childcare entry so far; see Ahnert et al., 2004; Bernard et al., 2015) reacts slowly by releasing pulses of cortisol with delays of 20 to 30 minutes, RSA changes promptly in response to environmental challenges and thus can be studied in relation to behavioral patterns associated with specific situations at given times. This paper explores children's RSA at arrival to the childcare centers, in the group, and alongside isolated play during three critical phases during childcare entry: (1) adaptation with mother present, (2) first separation day, and (3) the subsequent separation phase. In high-quality care centers, we expect children to cope with increasing RSA (lowering stress) at the end of the transition from home to child care. Whereas RSA in the group might remain challenged (low RSA), RSA during isolated play should become comparable with the children's RSA upon arrival at the center.

1.2 | The buffering function of attachment when children are under stress

There is broad consensus that children's care experiences shape the coping and regulation of stress (see Aldwin, 2007). From the perspective of attachment theory, children who have secure attachments respond to novel environments on the basis of trust in their care givers' availability and capacity to protect (e.g., Bowlby, 1969; Bretherton & Munholland, 2016). These features of secure attachment are believed to be retained in the children's memory and are known to buffer stress (e.g., Gunnar & Quevedo, 2007). In agreement with this perspective, research has documented attachment security-related differences in children's RSA (Smith et al., 2016). Whereas securely attached children's RSA remained high and stable over time, insecure children showed remarkably inconsistent RSA in that study. Other researchers (e.g., Hane & Fox, 2016; Spangler & Grossmann, 1993) have reported similar results in the classic strange situation procedure described by Ainsworth et al. (1978) .

We expected low RSA in infants who were securely attached prior to enrollment based on studies of mother–child separations at childcare entry that have illustrated the effectiveness of mothers in buffering their infants' stress levels. Research on children's cortisol responses (Ahnert et al., 2004) showed that infants who were accompanied by mothers to whom they were securely attached suppressed stress responses effectively. However, it was unclear whether this suppression was due to the presence and behavior of the mother or to the infants' memories of her prior behavior. Comparisons between children's RSA at arrival (when the mother is present) with RSA in the group or during isolated play (when the mother had left the child who could thus refer to either both memories of her previous behavior or to memories of her behavior immediately prior to her departure) allowed us to compare the effects of the mothers' immediate and more distant behavior on the infants' behavior.

1.3 | The role of care providers after children's separation from mother

Separation from parents is a major source of stress in infancy, and infants' separation protest is widely interpreted as an attempt to reestablish contact (Lummaa et al., 1998). The signal value of crying is increasingly shaped by caregiving experiences during the first year, especially when a sensitive care provider has established a secure relationship. Because the brainstem mechanisms regulating RSA are linked to facial expression and the prosodic structure of vocalizations (see Porges, 2011), we anticipated that low RSA would be correlated with greater protest behaviors. Thus, infants experiencing high stress levels (low RSA) would be more likely to express the negative emotions that are particularly important for children who are socially reticent and whose lower RSA baselines (Kagan & Fox, 2006) make them respond to stress faster. No matter what form it might take, children's cries after separation become a powerful means of soliciting aid and assistance from others (e.g., Zeifman, 2001).

In group settings, it is important to regulate cry-fuss bouts immediately in order to avoid peers contagiously imitating their negative emotions. However, there is also a growing corpus of research focused on the role of care providers and their reactions to infants in group care. Researchers have inquired about the processes through which care providers build relationships with children in group contexts (Ahnert, 2021; Ereky-Stevens et al., 2018; Ishimine & Tayler, 2014; van Schaik et al., 2017) and it has been documented that care provider–child relationships infrequently achieve the more positive qualities of child–mother relationships, even though care providers are as sensitive as mothers are (e.g., Ahnert et al., 2006; Beckh & Becker-Stoll, 2016; van Polanen et al., 2017). We therefore expected that infants

who displayed negative emotions would elicit more attention from and be offered more help by the care providers in the centers, which in turn would increase the likelihood that the infant–care provider attachments would become secure, thereby allowing them to help the children regulate stress.

1.4 | Research aims

By exploring infants' cardiac responses at childcare entry, we sought to determine whether the transition to childcare and the associated separations from parents cause a stressful physiological adjustment that is reflected in lower RSA. We further investigated whether infants' stress responses to childcare entry would be buffered more effectively in infants with secure infant–mother attachments and whether infants who expressed negative emotions in response to childcare entry triggered care providers to help the infants downregulate stress levels, thereby promoting the development of secure relationships with the care providers.

2 | METHOD

2.1 | Participants

Seventy healthy full-term infants (36 girls; 52 firstborns) averaging 14.9 months (SD = 1.7) of age were recruited in Berlin, Germany. The infants were born in families representative of Berlin's middle class with respect to parental age, education, occupation, and income (according to the sociological microcensus of Berlin, Germany). Before enrollment in childcare, all infants had been cared for at home, primarily by their mothers. Thirty-seven childcare centers with high-quality ratings provide care to these infants.

To ease the adjustment to group care, mothers were encouraged to accompany infants and toddlers during their first few days in the childcare centers. Group sizes ranged from four to six infants per care provider. The care providers involved were female, came from middle-class backgrounds, and had attended specialized three-year training courses at a medical college.

2.2 | Procedures and measures

2.2.1 | Overall procedure

The families were visited twice during the week before they entered childcare. The home visits provided opportunities to obtain information, including parental reports of infant temperament and measures of cardiac activity (home baseline). When childcare began, the infants attended part-time and were accompanied by their mothers for approximately two weeks (M = 9 days, SD = 6.9). Hours of attendance increased from two to four hours over the two weeks. Following this adaptation phase, all infants were enrolled for approximately 40 hours per week. The infants were videotaped, and their heart rate patterns were recorded on the first and last of the adaptation days (*First Day*, *Last Day*) during which their mothers were present, on the subsequent first separation day (*D1*), on the fifth and ninth days that the mothers were absent (*D5*, *D9*), and on one day five months later (*M5*) as follow-up. Ainsworth and Wittig's (1969) Strange Situation was used to assess infants' attachments to their mothers one week prior to childcare entry and to their primary care providers about five months later.

TABLE 1 Distribution of attachments to mothers and care providers (n = 64)

	Infant–care provider attachments		
	Secure	Insecure	Total
Infant–mother attachments			
Secure	13 (20%)	17 (26%)	30 (46%)
Insecure	12 (19%)	22 (35%)	34 (54%)
Total	25 (39%)	39 (61%)	64 (100%)

2.2.2 | Attachment assessments and classification

Infants were videotaped in Ainsworth and Wittig's (1969) Strange Situation with their mothers prior to childcare entry. Five months later, all but six were again observed in the Strange Situation with the care providers in the role of "mothers." Using Ainsworth's system (Ainsworth et al., 1978) and Main and Solomon's (1990) ratings, the first author and her assistant (classification agreement ≥ 90%) classified the infants as having *secure* [B], *avoidant* [A], *resistant-ambivalent* [C], and *disorganized* [D] attachments.

For purposes of the present analyses, however, [A], [C], and [D] patterns were grouped into one *insecure* category whereas [B] patterns were classified as *secure*. Of the 70 infant–mother attachments, 34 (49%) infants were labeled secure, whereas 36 (51%) were rated insecure with 32 (46% of the total; 89% of the insecure dyads) insecure-avoidant infants as in other German studies (Gloger-Tippelt et al., 2000). When we reassessed the infants with their care providers, six children were unavailable for a variety of reasons, even though they did not differ with respect to age, sex, and temperament from the other 64 infants. Table 1 summarizes the ratings of both infant–mother and infant—care provider attachments. Many (61%) of the infant—care provider attachments were rated in the insecure-avoidant [A] category. None of the results reported below changed when the other insecure (C and D), and nonattached dyads were removed from the analyses.

2.2.3 | Cardiac data collection and quantification

During the home visits, cardiac activity was recorded to familiarize infants and mothers with the procedure using "happy face" stickers to cover the ECG electrodes placed on the infants'

chests. Cardiac activity was recorded for approximately 5 minutes (M = 4.32 min, SD = 1.44) while mothers held the infants on their laps and maintained the infants in a quiet behavioral state by entertaining them with picture books. During subsequent monitoring sessions, investigators (or the mother) placed electrodes on the infants as they arrived at the centers. Beat-to-beat heart rate data were stored on a Seca Sportronic 300 MED placed in a small bag worn by the infants while they were videotaped to ensure that cardiac and behavioral responses could be time-linked. To synchronize behavior and heart rate measures, we videotaped the moment when the Seca Sportronic 300 MED was switched on. Based on these videotapes, we later identified three discrete situations during which cardiac data were extracted: (a) *Arrival* lasted from 2.5 to 5 minutes (M = 3:37 min; SD = 2:41) and monitored infant heart rate responses from arrival at the center until the infant entered the group. During that time, mothers involved the infant in a manipulative task comprising a set of boxes to open, close, and otherwise manipulate. (b) In-group *Play* situations lasted between 5.75 and 8.5 minutes (M = 7:19 min; SD = 4:26), and included situations during which infants played in the group without displaying any negative expressions (i.e., while exploring without gross motor activities and/or while interacting in a friendly manner). Cardiac activity from *Play* situations was considered "non-stressed," and appeared at the end of most observations. (c) From the moment the infants entered the group until the beginning of a *Play* situation, cardiac activity was monitored during all *Group* situations (M = 27:39 min; SD = 7:07). The Seca Sportronic 300 MED detected the R-wave peaks in each cardiac cycle and timed the interval between successive heart beats. These heart periods (HP) were timed to the nearest millisecond. Two research assistants manually edited the HP data to eliminate artifacts after being trained and supervised by the fourth author (interrater reliability, 98%). RSA were then quantified using MXEDIT (Delta-Biometrics, Inc., Bethesda, MD), a software program based on algorithms developed by Porges and Bohrer (1990). The methodology employed in MXedit to quantify RSA sensitively indexes vagal regulation of the heart (Lewis et al., 2011). For each target infant, RSA was computed for the home visit (baseline), and for each of the three situations (i.e., *Arrival*, *Play*, and *Group*) on two days that the mothers were present (*First* and *Last Days*) and on four days that they were absent (*D1*, *D5*, *D9*, and in *M5*).

2.2.4 | Negative emotions

Infant negative emotions were coded from the videotapes in the *Group* situations, and were quantified by noting episodes of pronounced negative vocalization as well as facial and behavioral expressions of whining, crying, and screaming. These behaviors were marked on a second-by-second basis using a video coding software that preserved the temporal sequence and summed the total duration of negative emotions (Mangold, 2015). Six coders rated the tapes and maintained high levels of agreement, with Cohen's kappas ranging from .88 to .98. Later analyses used scores that referred the summed duration of negative emotions to the overall duration of the respective *Group* situation (% of the time) and were then arcsine transformed in order to correct the nonnormality of the distribution.

2.2.5 | Infant temperament

Infants' temperaments were assessed using the German version of Fullard et al.'s (1984) Toddler Temperament Scales (Weise & Ahnert, 1999); we concentrated on scales most likely to be associated with responsivity to novelty such as mood, activity, and intensity, and thus most likely to influence cardiac responses. These temperament scores were later z-transformed for statistical analyses.

2.3 | Data analysis

2.3.1 | Infant cardiac responses and changes in negative emotion throughout the transition

While utilizing all RSA data for maximal statistical power, a multilevel regression allowed us to disentangle the effects of the transition during the adaptation phase (*First Day*, *Last Day*), the first day of separation (*D1*), the remaining separation phase (*D5*, *D9*), and the follow-up (*M5*), as well as the effects of the situations (*Arrival*, *Group*, *Play*) and infants' attachment to mother and care provider on RSA levels. A repeated-measures ANOVA was used to detect changes in infants' emotions in the group context during the period of transition.

2.3.2 | Conceptualizing and exploring infant protest behaviors

Measures of infant negative emotions were used to assess infants' protest behaviors as a complex (latent) variable. We further examined the interplay between these protests and infants' attachment classifications and temperament in a mixture model. The mixture model combined a *Latent Profile Analysis* classifying the infants into two types of protesters according to their negative emotions, and a *Path Model* testing both regression and prediction paths to detect preconditions and consequences for these protesters. The following assumption led to the following regression path: Because protest behaviors are elicited by children's separations from their mothers, the protests should be affected by infant–mother attachments, with secure attachments leading to higher levels of protest behaviors during separation than when insecure infants are separated (Ainsworth et al., 1978).

The following assumptions led to three prediction paths to describe the consequences of infants' protest behaviors in childcare centers: (i) because infants' negative expressions are powerful social signals eliciting attention (see Zeifman, 2001), these protests might motivate care providers to help infants regulate their negative emotions. As emotion regulation is central to infants' attachment formation (see Zimmer-Gembeck et al., 2017), furthermore, infants' protests and care providers' efforts to regulate them should in turn support infant–care

provider attachments. Different levels of protest behaviors should thus predict subsequent infant–care provider attachments differently, with high levels contributing to infant–care provider attachment formation more effectively than low levels of protest. (ii) Secure infant–care provider attachments should predict less intense negative emotions at the end of the adaptation process. Thus, secure infant–care provider attachments should be associated with less negative emotions five months after childcare entry than insecure infant–care provider attachments. (iii) Infant temperament, like mood and intensity, might affect emerging infant–care provider attachments, too, with higher intensity and mood amplifying the attachment process (see Groh et al., 2017).

Whereas the regression and predictions paths were evaluated based on beta or μ coefficients ($p < .05$), the overall fit of the mixture model was assessed using a measure of relative entropy, *E*, with scores ranging from 0 to 1. Values closer to 1 indicate a more distinct classification of infant protests and of the predictive pathways (Celeux & Soromenho, 1996).

3 | RESULTS

3.1 | Infants' cardiac responses throughout the transition

To determine infants' stress levels and regulatory capacities throughout the transition, RSA levels were examined during adaptation and separation in three phases of daily childcare entry, i.e., *Arrival*, *Group*, and *Play*. Figure 1 shows RSA levels during the course of transition separately for infants' attachments toward the mother and care provider.

A multilevel regression using the R package lme4 (Bates et al., 2015) considered the nesting of the RSA levels due to assessments in multiple situations and at multiple times. Consistent with the research questions, we examined the effects of three relevant parts of the transition: (1) adaptation [*First and Last Day*], (2) the first separation day [*D1*], and (3) the separation phase [*D5 and D9*]. These contrasts used the follow-up [*M5*] assessment as a reference, as well as the effects of the situations (*Group* and *Play* with *Arrival* as reference) on infants' RSA levels. In addition, the model explored infants' attachments to *Mother* and *Care Provider*, as well as the interactions between these attachments and all parts of the transition while controlling for baseline RSA, child's age, and gender. The overall model explained 24.4% of the RSA variance according to *R*š for generalized linear mixed-effects models (Nakagawa & Schielzeth, 2013).

Results showed that RSA significantly decreased during the adaptation phase, $b = -0.15$, $SE = 0.04$, $\beta_y = -0.20$, $p < .001$, and on the first separation day, $b = -0.25$, $SE = 0.05$, $\beta_y = -0.33$, $p < .001$. However, RSA was not lower during the subsequent separation phase than at the time of the 5-month follow-up. Furthermore, in the *group* infants displayed lower RSA than at *arrival*, $b = -0.05$, $SE = 0.02$, $\beta_y = -0.06$, $p = .041$, where the RSA was not different from that in the later *play* situations.

Interestingly, infants who developed secure attachments toward their care providers appeared, in general, to have lower RSA, which might reflect greater physiological disruption or stress than that experienced by infants with insecure attachments, $b = -0.31$, $SE = 0.10$, $\beta_y = -0.41$, $p = .002$. Interactions between attachment category and the different phases of the transition revealed reduced RSA levels during the separation phase ($b = -0.11$, $SE = 0.06$, $\beta_y = -0.15$, $p = .04$) but not on the first separation day or during the adaptation phase, during which there were greater individual differences in response profiles. Reduced RSA values were also evident as a result of interactions between secure infant–mother attachments and the separation phase, $b = -0.16$, $SE = 0.05$, $\beta_y = -0.22$, $p = .003$ whereas during the adaptation the opposite was true, $b = 0.27$, $SE = 0.05$, $\beta_y = 0.35$, $p < .001$, suggesting less stress when the mothers of securely attached infants were present (see Table 2 for all results).

3.2 | Infants' cardiac responses and changes in negative emotions

Infants' negative emotions were associated with RSA levels on the first separation day with infants who cried more having lower RSA, $r(69) = -.28$, $p < .05$. To determine how negative expressivity was associated with infant–care provider attachment, we subjected infants' negative emotions during the separations to a repeated-measures ANOVA with days [D1, D5, D9, and M5], and infants' attachment to care provider [secure vs. insecure] as independent variables. Attachment to care provider, $F(1, 62) = 5.02$, $p < .05$, $d = 0.57$, and separation days, $F(3, 60) = 13.76$, $p < .001$, $d = 1.66$, were significantly related to infant negative expressivity. Most remarkably, negative emotions were more common when infants were securely attached to care providers. Furthermore, a significant interaction between attachment to care provider and separation days, $F(3, 60) = 3.22$, $p < .05$, $d = 0.80$, suggested decreasing negative emotions over time when infant–care provider attachments were secure (see Figure 2). In sum, the findings suggested that the infants who protested more appeared more stressed initially (low RSA levels), showed larger RSA increases over time and were more likely to form secure attachments with their care providers.

3.3 | Infant protest behavior as related to attachment experience and temperament

To shed further light on infants' negative emotions, their preconditions, and their consequences, a mixture model was conducted which combined a *Latent Profile* with a *Path* model (Muthén & Muthén, 2012). Measures of negative emotions during separation (D1, D5, and D9) were first used for the *Latent Profile* model to segregate different levels of protest behaviors into two types of protesters. Of all infants, 35.3% protested at high levels and 64.7% at significantly lower levels (see Figure 3).

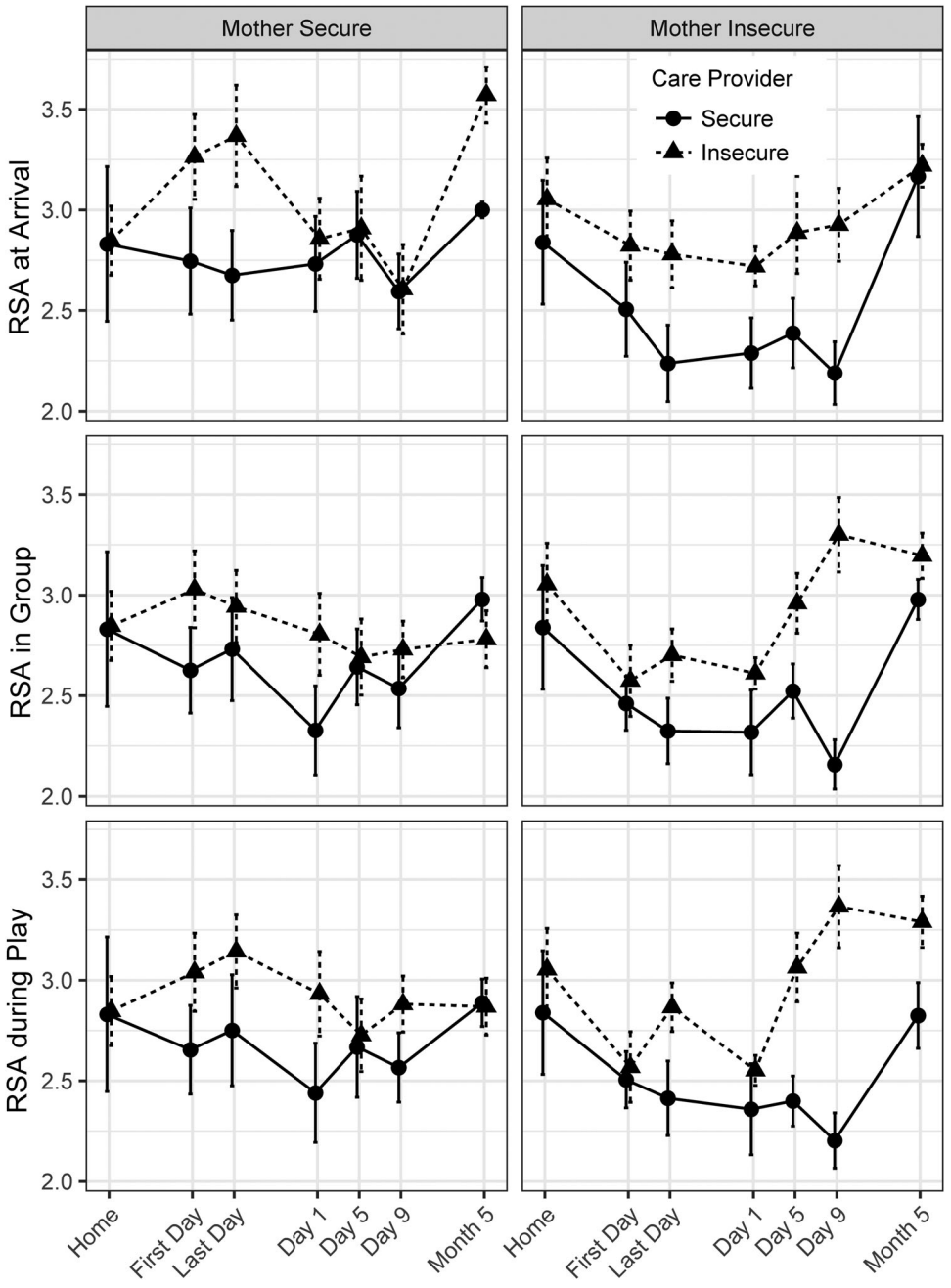

FIGURE 1 RSA levels at arrival, in the group, and during play in secure vs. insecure infant–mother and infant–care provider dyads throughout the transition to childcare

Path modeling explored regression and prediction pathways with infants' attachments, temperament, and the status of negative emotional expression at the end of the transition. The high-level protesters were associated (with $b = 1.22$, $OR = 3.38$, $p = .041$) with secure infant–mother attachments as well as with secure infant–care provider attachments at month 5 ($\mu = -2.45$, $p = .012$), suggesting that high protesters were more likely than low protesters to form secure relationships with their care providers.

Furthermore, secure infant–care provider attachments at month 5 were associated with lower levels of negative emotions at the end of the transition ($b = -0.30$, $p = .007$). With regard to infant temperament, only intensity had an effect on infant–care provider attachment ($b = -1.15$, $p = .030$). Whereas high intensity appeared to weaken the formation of infant–care provider attachments, mood did not have an effect ($b = 0.46$, $p = .195$). Thus, intense temperaments might hinder the formation of secure relationships with care providers, and might be more elevated for the insecure than for the secure infant–care provider dyads. Overall, the mixture model demonstrated an excellent *relative entropy E* of .95; for an overview see Figure 4.

TABLE 2 Results of multilevel regressions on infants' RSA

	b	β_y	*SE*	*t*(df)	*p*
Intercept	2.26	−0.68	.32	7.0 (64.1)	>.001***
Transition (reference: *M5*)					
Adaptation (*First/Last Day*)	−0.15	−0.20	0.04	3.6 (1087)	>.001***
First separation day (*D1*)	−0.25	−0.33	0.05	4.7 (1087)	>.001***
Separation phase (*D5/D9*)	0.08	0.10	0.04	1.9 (1087)	.06
Situation (reference: *Arrival*)					
Group	−0.05	−0.06	0.02	2.0 (1087)	.04*
Play	0.00	0.00	0.02	0.1 (1087)	.94
Secure attachment to					
Mother	0.12	0.16	0.10	1.3 (66.1)	.20
Care provider	−0.31	−0.41	0.10	3.2 (66.2)	.002**
Secure attachment to mother X					
Adaptation (*First/Last Day*)	0.27	0.35	0.05	4.8 (1087)	>.001***
First separation day (*D1*)	0.11	0.14	0.07	1.6 (1087)	.12
Separation phase (*D5/D9*)	−0.16	−0.22	0.05	3.0 (1087)	.003**
Secure attachment to care provider X					
Adaptation (*First/Last Day*)	−0.03	−0.04	0.06	0.6 (1087)	.55
First separation day (*D1*)	−0.01	−0.01	0.07	0.1 (1087)	.92
Separation phase (*D5/D9*)	−0.11	−0.15	0.06	2.0 (1087)	.04*
Control Variables					
Baseline RSA	0.26	0.35	0.05	5.6 (64)	>.001***
Child is female	0.11	0.15	0.10	1.2 (64)	.24
Age in months	−0.02	−0.02	0.02	0.8 (64)	.42

Note. b = unstandardized regression coefficient; β_y = regression coefficient for standardized RSA; SE = standard error.
***p <.001; **p <.01; *p <.05.

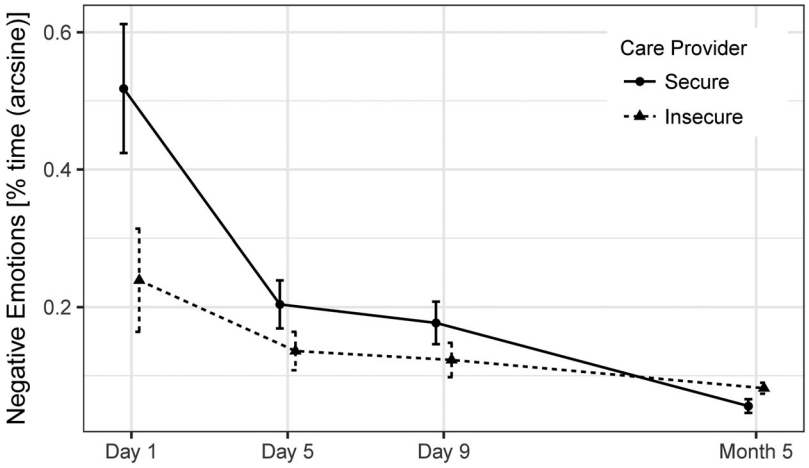

FIGURE 2 Negative emotions throughout the separation phase in secure vs. insecure infant–care provider dyads 322 × 199 mm (300 × 300 DPI)

4 | DISCUSSION

The present study provides further insights into infants' transitions from home to childcare and on the ways in which childcare entry affects infant psychobiological functioning. To begin with, our analyses of infant cardiac responses complement earlier analyses of cortisol levels, demonstrating that the onset of care is stressful (Ahnert et al., 2004). Mothers' presence early in the period of transition helped attenuate the stress, especially when infants were securely attached to them. Just as cortisol elevations were suppressed during the

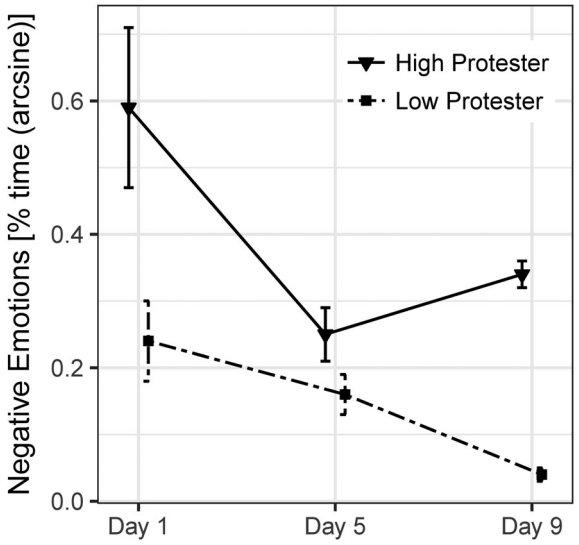

FIGURE 3 Levels of protest behaviors during the separation phase 199 × 199 mm (300 × 300 DPI)

adaptation period when mothers were present, higher RSA in secure infants showed that they experienced less stress at the onset of the transition to childcare. This stress attenuation continued during the first day of separation when secure infants could regulate biobehavioral state while anticipating the return of their mothers. However, the subsequent separation phase appeared to be particularly crucial in ALL infants' adaptation to childcare. When infants entered the group without their mothers, they expressed negative emotions most intensively, and RSA levels went down (just as cortisol levels rose; Ahnert et al., 2004). Children, however, later resumed play, and RSA normalized.

Expressions of negative emotions were greatest on the first day of separation, even though the infants were very familiar with the facilities and staff before the separations began. Infants expressed negative emotions longer when they were more distressed. Such associations suggest that the infants were appealing for a reunion with their mothers (Lummaa et al., 1998; Zeifman, 2001). More than one-third of the infants fussed and cried extensively (high protesters), especially on the first separation days, although negative emotions became progressively less prominent over the following weeks and appeared minimal at the end of the transition. These findings are not consistent with those reported by Fein and her colleagues (Fein, 1995; Fein et al., 1993), who observed despair-like behaviors as much as 6 months after enrollment in high-quality childcare in Italy. Because the cultures and situations differed, it is difficult to distinguish among several possible reasons for the observed differences. As others have reported (e.g., Ahnert et al., 2000), displays of negative emotion by children in childcare are common for a variety of reasons.

The design of the present study allowed examination of the circumstances during which infants cried and the ways in which those negative expressions become functional in the new care environments. The factors associated with negative expressivity were infants' stress levels and regulatory capacity as well as their experiences of responsive care as measured by the security of attachment. High levels of manifest stress (low RSA) indicate low regulatory capacity. Whereas secure attachment should be associated with better regulatory capacity, insecure-avoidant attachment (which predominated in this as in other German samples) leads infants to cope with the stress on their own (Hane & Fox, 2016). Insecure-avoidant infants tend to hide negative emotions and thus cope silently and these infants were found in the low-protest group. They actively avoided proximity and contact with the care provider and were less likely to develop secure relationships with them. When the few expressions of distress were overly intense, the formation of infant—care provider attachments was further obstructed. Consequently, insecure infants with low regulatory capacities risk long-lasting stress exposure whereas insecure infants who displayed mature regulatory skills managed well during the transition to childcare. In contrast, infants who were securely attached to their mothers displayed more negative emotions (high protesters) and were more likely to develop secure relationships with the care providers who helped them cope with the transition. This clearly suggests that negative emotional displays by the infants brought about stress regulating interventions by facilitating communication with caretakers, especially at ages when verbal signals were not yet possible (Zeifman, 2001). Initially high levels of negative emotion were predictive of secure infant–care provider attachments, which, once developed, reduced the levels of negative emotions.

In sum, the regulatory effects of mother–child interaction that were disrupted by maternal separation inevitably created stress with which

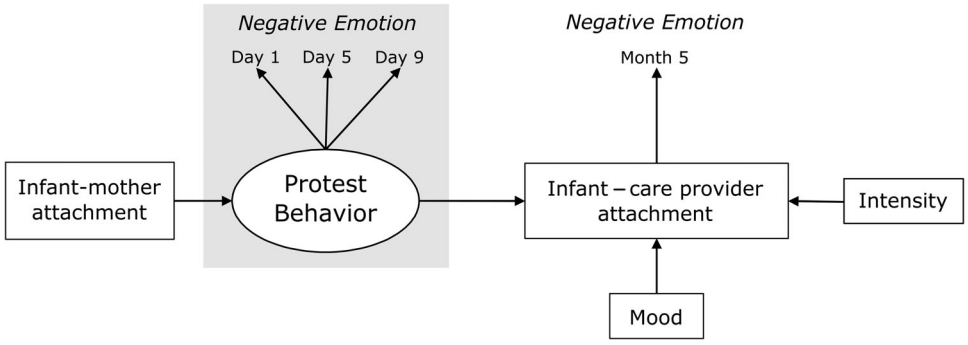

FIGURE 4 Mixture model to describe infants' protest behaviors, and its preconditions and consequences during the transition to childcare 515 × 179 mm (300 × 300 DPI)

the infants coped either on their own (as the insecure infants did) or with the assistance of others. Secure attachments to care providers were thus fostered by stressful circumstances, although such attachments subsequently helped the infants to regulate stress. The data reported here suggest that infants can regulate stress in the absence of their mothers when given sensitive substitute care, even developing regulatory competence in such circumstances.

During mother–child separations, care providers must help to downregulate stress levels, especially in infants who protest extensively and only want their mothers back. Because extensive protest seems to indicate limited regulatory capacities, infants risk overburdening the stress system when left unsupported. Although it seems reasonable to expect that peak stress occurs when children are dropped off at childcare, we did not determine whether there were increased levels of stress (as indexed by RSA) over the course of the day. Alternatively, dynamic changes in RSA during the transitory disruption and recovery in autonomic state over hours and days of mother–child separation, rather than indexing stress burden might may provide a portal to study the development of resilience. This limitation of the study should be overcome by further research involving multi-hour and multi-day heart rate monitoring.

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AUTHORS' CONTRIBUTION

Lieselotte Ahnert made substantial contributions to conception and design and interpretation of data; she also drafted the MS and revised it during the revision process after discussions with all authors. Tina Eckstein-Madry reworked data files, prepared data analysis and discussed results. Bernhard Piskernik provided the data modeling and interpretation of data. Stephen W. Porges taught about the cardiocirculatory system and supervised the handling of the heart rate data. Michael E. Lamb initiated the project, and supervised the conception, design and interpretation of data. All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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