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Solitary sleeping in young infants is associated with heightened cortisol reactivity to a bathing session but not to a vaccination

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KEYWORDS Cortisol; Stress; Reactivity; Sleep; Co-sleeping; Infant; HPA-axis; Sensitivity; Breastfeeding	 Summary Background: In this prospective longitudinal study, we investigated the relation between sleeping arrangements and infant cortisol reactivity to stressors in the first two post-natal months. Cosleeping, as compared to solitary sleeping, is hypothesized to provide more parental external stress regulation by night, thus reducing general stress sensitivity. We therefore expected lower cortisol reactivity to stress in infants who co-slept more regularly. Methods: Participants were 163 mothers and infants from uncomplicated, singleton pregnancies. Mothers completed daily diaries on sleeping arrangements in the first 7 weeks of life. Co-sleeping was defined as sleeping in the parents' bedroom (i.e. own or parents' bed). Cortisol reactivity was measured twice: to a mild physical stressor (bathing session) at 5 weeks of age and to a mild pain stressor (vaccination) at 2 months of age. Results: Infants with a solitary sleeping arrangement in their first month of life showed a heightened cortisol response to the bathing session at 5 weeks compared to infants that coslept regularly. This effect was not explained by breastfeeding practices, maternal caregiving behavior, or infants' night waking and sleep duration. No effects were found of co-sleeping on the cortisol response to the vaccination at 2 months. Conclusions: The results suggest that solitary sleeping in the first month of life is associated with heightened sensitivity of the HPA-axis to a mild stressor, possibly due to less nocturnal parental availability as external stress regulator. Whether this effect continues in later life, remains to be
	availability as external stress regulator. Whether this effect continues in later life, remains to be investigated. © 2011 Elsevier Ltd. All rights reserved.

Abbreviation: HPA-axis, hypothalamic—pituitary—adrenal axis. * Corresponding author at: Department of Developmental Psychology, Behavioural Science Institute, Radboud University Nijmegen, PO Box 9104, 6500 HE Nijmegen, The Netherlands. Tel.: +31 24 3612637; fax: +31 24 3615937.

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1. Introduction

In the present study we focus on the relation between parent—infant nocturnal sleeping arrangements in early infancy and infant cortisol reactivity to acute stressors. In

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Western countries infants' sleeping arrangements during the first months of life show large inter-individual variation: while some infants sleep in their own room from the beginning, others sleep in a crib in the parents' room, and yet others sleep in bed with the parents. These last two options are commonly referred to as 'co-sleeping' (Goldberg and Keller, 2007a; McKenna et al., 2007).

An area that has been largely unexplored is that of the relation between early sleeping arrangements and infant stress reactivity. In response to stressors the human body reacts with the release of glucocorticoids, mainly cortisol, by the hypothalamic-pituitary-adrenal (HPA) axis. The HPAaxis starts to develop prenatally, but fully matures after birth (Lupien et al., 2009). That is, in the first year of life, basal cortisol levels slowly decrease (Tollenaar et al., 2010), and a circadian rhythm starts to develop within a few months after birth (de Weerth et al., 2003). Cortisol reactivity to stressors is found early after birth, but seems to diminish after about 6 months (Gunnar et al., 2009b; Jansen et al., 2010a). The HPAaxis is found to be shaped by early environmental factors like parental care, parental separations, or early life stress (e.g. neglect or abuse; Gunnar and Donzella, 2002; Elzinga et al., 2008; Gunnar et al., 2009a). Sleeping arrangements may constitute an early environmental factor that can influence the HPA-axis, as it relates to the proximity of parents at night. Given that dysregulation of the HPA-axis can be a risk factor for the development of (psycho)pathology (Heim et al., 2000; Gunnar and Vazquez, 2001; Lupien et al., 2009), it is important to investigate whether and how early sleeping arrangements are associated to infant HPA-axis functioning.

In the first postnatal months an infant's self-regulation capacities are developing quickly but are as yet limited. Therefore, parents have an important role as external regulators of distress levels of the child, e.g. by sensitively responding to the infant's signals and needs (Haley and Stansbury, 2003; Hofer, 2006; Albers et al., 2008). Co-sleeping implies more physical closeness to the parents during the night compared to solitary sleeping, making parents more physically available, and more quickly available, to help the infant regulate distress. The nocturnal parental separation for solitary sleepers probably means that infants' subtle signals of discomfort are less, or more slowly, responded to by parental vocalizations and/or touch than for co-sleeping infants. Solitary sleeping may thus be related to more frequent experiences of higher levels of distress (and presumably higher cortisol levels) during the night, as the infant will probably have to reach higher levels of negative vocalizations in order to alert the parents. This may sensitize the HPA-axis, leading to heightened cortisol reactivity to stressors during the day as well (Heim and Nemeroff, 2001). Support for this hypothesis comes from animal research showing that early maternal separations are a large contributor to the development of the HPA-axis. Daily dam-rat separations of 3 h or more have been associated with a hyper-responsive HPA-axis (including higher corticosterone levels after stress: Meaney et al., 1996; Aisa et al., 2007, 2008), while increased maternal caregiving behaviors are related to lowered corticosterone responses to stress (Liu et al., 1997). The current paper will thus investigate whether solitary sleeping is related to higher cortisol reactivity to stress compared with co-sleeping.

Sleeping arrangements may, however, be associated with certain parental and infant factors that can influence infant

cortisol reactivity to stress as well. Hence, these factors may provide alternative explanations for a relation between cosleeping and cortisol reactivity. Several factors may be of special interest for the current paper and will shortly be discussed. Breastfeeding, for example, is a major reason to co-sleep (McKenna et al., 1997; Ball, 2003), and may by itself also influence cortisol levels (Waynforth, 2007; Cao et al., 2009). The quality of maternal caregiving behavior is another factor that may be associated with both the choice to cosleep (Taylor et al., 2008), and the mother's abilities to help the infant regulate stress. For example, Albers et al. (2008), and Haley and Stansbury (2003) found a relation between mothers' sensitivity during caregiving and cortisol regulation in their 3-month-old infants (note however that Jansen et al., 2010b, found no such relations at 5 weeks after birth). Another factor related to co-sleeping may be infant sleep characteristics in the form of night waking and sleep duration (Hunsley and Thoman, 2002; Cortesi et al., 2004; Mao et al., 2004). These sleep characteristics may also influence cortisol reactivity or levels during the day (Lucas-Thompson et al., 2009; Scher et al., 2010) and were therefore included in the study. In sum, in the current study we controlled for the effects of breastfeeding, maternal caregiving behavior, and infant sleep characteristics, by testing whether they were related to co-sleeping and if so, whether co-sleeping explained any additional variance in cortisol reactivity after adjusting for these factors. We also controlled for several other infant and maternal factors (e.g. maternal age and education, number of siblings, infant birth weight).

To our knowledge, only two human studies investigated the relation between parent-infant sleeping arrangements and HPA-axis functioning. One study looked at basal cortisol levels (Waynforth, 2007), and the other at cortisol reactivity (Lucas-Thompson et al., 2009). Waynforth found that fewer years of co-sleeping was related to higher basal cortisol levels in British 3- to 8-year-old children, which is in line with the hypothesis that solitary sleeping may lead to heightened HPAaxis activity. However, Waynforth's study sample constituted a fairly heterogeneous age group with retrospectively collected co-sleeping data, and no cortisol reactivity data. Lucas-Thompson et al. (2009) did collect cortisol reactivity data to vaccinations at 6 and 12 months of age. They found that current co-sleeping, as reported in a maternal questionnaire over the previous month, was associated with increased cortisol reactivity. Summarizing, the very few studies on the relation between co-sleeping and HPA-axis functioning yielded conflicting results, did not use very detailed measures of co-sleeping (i.e., retrospectively or with a questionnaire), and did not focus on cortisol reactivity in the first months after birth.

The present study is the first to investigate the relation between parent—infant sleeping arrangements and cortisol reactivity during the first two post-natal months, a period in which availability of the parents as external stress regulators may play an important role in the development of the HPAaxis. Moreover, the study used co-sleeping data that were based on 7 weeks of daily diary data on sleeping arrangements, and assessed cortisol reactivity to two stressors. These two stressors were a home bathing session at 5 weeks of age (i.e. a mild physical stressor) and the routine Well Baby clinic vaccinations at 8 weeks of age (i.e. a mild pain stressor). Both stressors are known to elicit reliable cortisol responses in the first months of life (Albers et al., 2008; Gunnar et al., 2009b; Jansen et al., 2010a).

In sum, in this paper we examined nightly co-sleeping in the first months after birth in relation to infants' cortisol reactivity to stress. Co-sleeping, as compared to solitary sleeping, is hypothesized to provide more parental external stress regulation by night, thus reducing the infant's general stress sensitivity. We therefore expected lower cortisol reactivity to stress in infants who co-slept more regularly.

2. Methods

2.1. Participants

This study is part of an ongoing prospective longitudinal project on the role of early caregiving factors in infant development. Participants were healthy women living in the Netherlands, who were recruited during pregnancy through midwife practices. The study was approved by the university ethical committee for behavioral sciences and written informed consent was obtained from each participant at enrollment.

Inclusion criteria were: uncomplicated, singleton pregnancy, clear understanding of the Dutch language, no drug use, and no current physical or mental health problems. Of the 220 women that originally enrolled, 20 were excluded because of medical reasons such as preterm birth, major birth complications and drug-use during pregnancy. Of the remaining 200 mothers, information on parent-infant sleeping arrangements during the first 2 months of life was collected by 173 mothers. Main reasons for not participating were lack of time, lack of interest, or other private circumstances. For 163 of these 173 mothers, at least one valid cortisol sample was collected during the bathing session (N = 137) or the vaccination (N = 142). Missing cortisol data were due to time and scheduling problems, technical problems (e.g. not enough saliva, or sample timing problems), or outliers (see statistical analyses). These 163 mothers and their infants constituted the study population for the present study. All infants (90 boys, 73 girls) included in the project were healthy, born at full term (\geq 37 weeks) and had a 5 min APGAR score \geq 7. Demographic characteristics and study variables of the mothers and infants are provided in Table 1. The mothers in the study population were slightly older than the other 37 women from the overall group of 200 mothers (32.7 years and 31.3 years, respectively, F(1, 198) = 4.58, p < .04). They did not differ on any other infant or maternal factors (all ps > .05).

2.2. Procedure

In the last trimester of pregnancy (M = 37.7 weeks, SD = 1.84) the mothers filled in questionnaires on demographics. They also received instructions and materials for the sleeping arrangement diary that would start directly after birth. The sleep diary is explained below.

After we received notice that the mothers had delivered, they were contacted by phone to schedule a home visit when the infants were approximately five weeks of age (M = 33.5 days, SD = 4.9). During the home visit, we asked the mothers to bathe their infant as they would normally do (undressing, bathing, and dressing). The bathing sessions lasted on average 11.2 min (range: 6–20 min). We collected infant saliva to measure infant cortisol reactivity to the bathing session. The bathing sessions were also videotaped and later rated for quality of maternal caregiving behavior.

At around 2 months of age (M = 62.7 days, SD = 6.9) the infants received their first routine vaccinations at the Well Baby clinic. The vaccination included two injections; the first was a combined vaccination for diphtheria, whooping cough, tetanus, poliomyelitis and haemophilus influenzae type b, the second for pneumococcus. Before the vaccinations the babies also received a physical exam. Cortisol responses to this vaccination procedure were measured. Mothers collected the saliva samples themselves.

2.3. Instruments and measures

2.3.1. Sleeping arrangements and sleep characteristics

Information on parent—infant sleeping arrangements was collected with the use of daily sleep diaries in the first two months of life. Every morning the mothers filled in a diary on how long and where the child had slept during the previous night. They could mark this with lines in a table that consisted of 30-min time blocks spanning between 0000 h and 0800 h. They could indicate for every time block whether the child slept in its own room, in the parents' room (in a separate bed), in the parents' bed, or somewhere else. When the child was awake, no line was drawn for that 30-min block.

Table 1 Overview of demographic characteristics and study variables of the participating mothers and infants (*N* = 163).

	Mean (SD)	Range
Birth weight (g)	3599 (468)	2645-4730
Number of siblings	0.75 (0.7)	0—2
Number of breast feedings per day in the first month	5.7 (3.0)	0—12
Number of breast feedings per day in the second month	4.6 (3.1)	0—12
Maternal age at birth (years)	32.7 (3.7)	21.1-42.9
Percentage highly educated mothers (College or University)	78	
Percentage that reported smoking during pregnancy	3.1	
Percentage that reported alcohol use during pregnancy	14.1	
Maternal caregiving behavior (sensitivity and cooperation) at 5 weeks	5.3 (2.0)	1—9
Time of day of bathing session	1220 h (0200 h)	0920 h—1700 h
Time of day of vaccination	1150 h (0215 h)	0855 h—2055 h



Figure 1 Distribution of parent-infant co-sleeping (a) in the first 4 weeks of life, and (b) in the first 7 weeks of life.

In the same diary, mothers marked every time the infant woke during the night and required comforting to settle back to sleep. The percentage of time spent in each sleeping arrangement per night was calculated by adding up the number of sleeping blocks for each sleeping arrangement separately, and dividing by the total number of sleeping blocks for that night, multiplied by 100. Weekly averages were calculated for the total amount of hours slept per night, the percentage of time spent in each sleeping arrangement, and the number of night wakings, when at least 4 out of 7 days had been filled in.

In the first two months about half of the infants slept at least half the night in their own room or in a separate bed in their parents' room, and only 5% of the infants slept more than half the night in their parents' bed. As we considered this last group too small to analyze separately, we classified co-sleeping as sleeping in the parents' room, including both sleeping in a separate bed and in the parents' bed.¹

The bathing sessions were scheduled in week 5, so we calculated average co-sleeping in the first 4 weeks from the weekly averages. The vaccinations were scheduled at 2 months of age and therefore average co-sleeping arrange-

ments of the first 7 weeks were used to calculate co-sleeping for those analyses. For analyses on cortisol reactivity to the bathing session participants were only selected if at least 3 out of 4 weeks of co-sleep data were available, and for the vaccination analyses when at least 4 out of the 7 weeks of cosleep data were available (N = 155 and 159 out of 163, respectively).

The distributions of the average percentages co-sleeping per night in the first 4 and 7 weeks are shown in Fig. 1a and b. It is clear from this figure that co-sleeping is not normally distributed. For the present study we divided the infants into 3 groups: solitary sleepers (co-sleeping 0-10% of the time), full co-sleepers (91-100% of the time), and a middle group of 'partial' co-sleepers (11-90% of the time). For the 4-week analyses the groups were divided as follows; solitary sleep: N = 38, partial co-sleep: N = 45, full co-sleep: N = 72, and for the 7-week analyses; solitary sleep: N = 44, partial co-sleep: N = 54, and full co-sleep: N = 61. For the analyses, 2 dummy variables were created to compare the 3 groups. The first dummy variable indicates the contrast of partial and full cosleepers versus solitary sleepers (the Solitary sleep dummy: solitary sleeping = 0, partial and full co-sleeping = 1), and the second dummy variable indicates the contrast of full cosleepers versus solitary and partial co-sleepers (the Full Co-sleep dummy: solitary sleeping and partial co-sleeping = 0, full co-sleeping = 1).

¹ Analyses without the bed-sharers gave similar results.

2.3.2. Cortisol

Infant saliva samples were collected using Sorbette eye sponges. Samples were taken at arrival of the researcher to the home or of the parents to the Well Baby clinic (T1), and at 25 min (T2) and 40 min (T3) post-stressor (being taken out of the bath and receiving the vaccinations, respectively). After the stress sessions, samples were kept in the freezer (-18 to -25 °C) until further analysis. The saliva samples were analyzed with radioimmunoassay at the Laboratory of Endocrinology of the University Medical Center of Utrecht University. The lower limit of detection was 1 nmol/L, and inter-assay and intra-assay variations were below 10% (for details see de Weerth et al., 2007).

2.3.3. Confounders

Potential confounders that were measured in this study were maternal quality of caregiving behavior, breastfeeding, infant night wakings and sleep duration. Similar to Albers et al. (2008), the videotaped bathing routines were rated for maternal quality of caregiving behavior, including measures of sensitivity and cooperation (Ainsworth et al., 1978). Sensitivity refers to the extent to which caregivers timely and appropriately respond to the infant's needs and signals, and *cooperation* refers to the extent to which caregivers adjust their behavior to the infant's ongoing activity rather than interfering with the infant's actions. Sensitivity and cooperation were scored using two 9-point rating scales, with higher scores reflecting more sensitivity and cooperation. Interactions were rated separately by at least two trained students, who did not know the mothers and infants they were observing, and were blind with regard to the other data. Inter-observer reliability was very good (Cohen's Kappa: 0.90 for both sensitivity and cooperation).

Each week the mothers noted the average number of breast or bottle feedings per day in the sleep diary. The weekly average number of wakings and sleep duration per night were determined from the daily diaries, as described above. From these weekly means, we calculated the average number of daily breastfeedings, night wakings and average sleep duration for the first and second month separately.

In addition, we also included the following child and maternal variables as potential confounders, as these might all be related to co-sleeping and/or influence HPA-axis functioning: gender, birth weight, parity, maternal educational level, maternal age, pregnancy smoking (yes or no), pregnancy alcohol intake (yes or no), and time of day of the stressor.

2.4. Statistical analyses

Cortisol values higher than 3 *SD* from the group mean per sample moment were regarded as outliers and treated as missing values (2.5%). As cortisol scores were not normally distributed, a square root and logarithm transformation were performed on the bath and vaccination session data, respectively, and these transformed variables were used for the analyses. In the results section untransformed data are presented.

First, paired sampled *t*-tests were performed to test cortisol reactivity to the stressors. Next, ANOVAs and *t*-tests were used to examine differences between the groups on

each of the potential confounders. Then, to study the relations between co-sleeping and cortisol reactivity to the bathing session and the vaccination, we performed longitudinal regression analyses using mixed-model (multi-level) designs in SPSS 15.0. A major advantage of multilevel modeling over repeated measures analyses is the potential to include infants with missing data at one or two of the time points. With this technique, all valid data points could be included in the model. Time (sample moments T1, T2 and T3) was introduced at level 1 and nested within the individuals (level 2). Time was considered a random factor. Besides time as a linear factor, time squared was entered as a fixed factor to account for the increase and decrease in cortisol over time.

This base model was compared to multiple additional models. First, we entered the potential confounding variables for which the co-sleep groups differed as fixed factors, as these could explain possible effects of co-sleeping on cortisol reactivity.² We also included the interactions between the time variables and these variables to test whether they affected cortisol levels over time. In subseguent models, we only included those confounders that were significantly related to co-sleeping, to examine whether cosleeping explained any additional variance in cortisol reactivity. We added the 2 dummy variables to code for the 3 cosleep groups. We also included the interactions between the time variables and the dummy variables to examine the effect of co-sleeping on cortisol reactivity over time. The final models were compared on the basis of their deviance on the -2 log likelihood ratio scale after Maximum Likelihood estimation. Finally, to disentangle possible effects of the cosleep (or other) variables on cortisol reactivity, we used post hoc one-way ANOVAs and t-tests to study group differences at each cortisol sampling time.

3. Results

3.1. Preliminary results

3.1.1. Cortisol reactivity

Being taken out of the bath resulted in a significant increase in cortisol concentrations from sample moment T1 to T2: 11.5-14.5 nmol/L (t(99) = 3.4, p < .001). Cortisol significantly decreased again from T2 to T3: 14.5-12.3 nmol/L(t(90) = 4.7, p < .001). Cortisol levels at T3 no longer differed from levels at T1 (t(89) = 1.1, p = .29).

The vaccination resulted in a significant increase in cortisol concentrations from sample moment T1 to T2: 10.8-17.1 nmol/L (t(102) = 5.6, p < .001). Cortisol significantly decreased again from T2 to T3: 17.1-15.6 nmol/L (t(92) = 4.0, p < .001). Cortisol levels at T3 were still higher than at T1 (t(95) = 4.1, p < .001).

See Fig. 2 for the average cortisol levels at the three sample moments, for each stressor and in each of the three co-sleep groups. The cortisol responses to the two stressors (T2 minus T1) did not correlate (r = .016, p = .89).

² In the interest of parsimony and to reduce type I errors, we only included confounders in the regression analyses that differed between the groups.



Figure 2 Cortisol levels (mean \pm SEM) in the solitary sleep, partial and full co-sleep groups (a) before, 25 min after and 40 min after the bathing session, and (b) before, 25 min after and 40 min after the vaccination. *p < .05, two-tailed.

3.1.2. Confounders

Because maternal sensitivity and cooperation were highly correlated (r = .83, p < .001), an overall quality of maternal caregiving behavior score was computed by averaging the scores on both scales. As breastfeeding in the first and second months were highly correlated (r = .88, p < .001), we averaged the number of daily breastfeedings in the first and second month for the mixed model analyses on the vaccination. See Table 2 for the averages of all confounders per cosleep group.

For the bathing session we compared the solitary, partial and full co-sleepers in the first 4 weeks on all potential confounders. The groups differed significantly on maternal education ($\chi^2(1) = 4.2$, p < .05) and breastfeeding in the first month (F(2, 148) = 6.8, p < .001), with full co-sleepers having higher educated mothers than solitary sleepers. Partial and full co-sleepers received more breastfeeding. No differences between the co-sleep groups were found on maternal caregiving quality, the sleep variables (duration and waking), or any of the other potential confounders. Hence, only education and breastfeeding were added to the bathing session multilevel models as control variables.

For the vaccination we compared the solitary, partial and full co-sleepers in the first 7 weeks on all potential confounders. The groups differed significantly on maternal education $(\chi^2(1) = 9.0, p < .001)$, maternal age (F(2, 136) = 3.1, p < .05), and on breastfeeding in the first two months (F(2, 151) = 4.62, p < .05; F(2, 153) = 9.3, p < .001), with full co-sleepers having the highest educated and oldest mothers, and partial and full co-sleepers receiving more breastfeeding than the solitary sleepers. No differences between the co-sleep groups were found on maternal caregiving quality, the sleep variables or any of the other potential confounders. Hence, only education, maternal age, and breastfeeding were added to the vaccination multilevel models as control variables.

3.2. The effects of co-sleeping on cortisol reactivity

3.2.1. The bathing session

We performed multilevel regression analyses on the cortisol reactivity to the bathing session. The confounding variables that differed between the co-sleep groups (i.e., maternal education and breastfeeding) were added to the model, as well as their interactions with time and time squared (see Table 3, model with confounders). Breastfeeding had no significant effect on cortisol levels over time (all ps > .50). The interactions of education with the time variables

Table 2 Means and standard deviations of the potentially co	onfounding variable	s for each co-sleep	group.			
	Co-sleeping in th	ie first 4 weeks		Co-sleeping in the	first 7 weeks	
	Non	Partial	Full	Non	Partial	Full
Percentage boys	55	51	54	55	56	54
Birth weight (g)	3654 (392)	3527 (518)	3603 (453)	3608 (400)	3527 (521)	3648 (458)
Number of siblings	0.61 (.68)	0.71 (0.76)	0.88 (0.71)	0.55 (0.66)	0.81 (0.78)	0.84 (0.69)
Number of daily breast feedings in the first month	4.4 (2.8)	5.7 (3.1)	6.5 (2.7)*	4.7 (2.9)	5.7 (3.1)	6.5 (2.7)*
Number of daily breast feedings in the first two months	I	Ι	I	3.9 (2.8)	5.11 (3.1)	6.1 (2.6)*
Maternal age at birth (years)	31.9 (3.8)	32.3 (3.6)	33.6 (3.7)	31.6 (3.9)	32.6 (3.1)	33.7 (3.9)*
Percentage highly educated mothers	71	72	88 [*]	67	70	92*
Percentage that reported smoking during pregnancy	c	2	4	2	6	2
Percentage that reported alcohol use during pregnancy	13	18	12	14	19	11
Quality of maternal caregiving behavior	4.8 (2.1)	5.6 (2.0)	5.4 (2.0)	5.0 (2.1)	5.3 (2.1)	5.6 (2.0)
Mean number of night wakings in the first 4/7 weeks	1.8 (.69)	1.8 (.91)	2.0 (.87)	1.6 (.64)	1.8 (.86)	1.9 (.81)
Mean total sleep duration per night in the first 4/7 weeks (h)	7.2 (.75)	7.2 (.82)	7.1 (.87)	7.3 (.72)	7.2 (.82)	7.0 (.85)
Time of day of bathing session	1220 h (0215)	1210 h (0155)	1230 h (0210)	1210 h (0255)	1230 h (0110)	1215 h (0210)
Time of day of vaccination	1220 h (0225)	1150 h (0215)	1135 h (0205)	1235 h (0215)	1145 h (0210)	1120 h (0200)
⁵ Significant difference between the co-sleep groups in the first	4 or 7 weeks ($p < .0$	J5).				

(linear and squared) were significant (B = -0.27, p = .02 and B = 0.12, p = .03, respectively). Post hoc test revealed that infants from higher educated mothers showed lower cortisol levels at 25 min after the bath (T2: F(1, 109) = 5.28, p = .02), but not before or 40 min after the bath (ps > .19). We entered the education variables into the next model.

Then we added the two dummy variables that classified the three co-sleep groups, as well as the interactions between time, time squared and the two dummy variables. The interactions between the time variables (linear and squared) and the Solitary sleep dummy (contrasting the solitary sleep group with the partial and full co-sleep group) showed significant effects (B = -1.25, p < .005 and B = 0.46, p < .05, respectively), indicating a larger cortisol reaction in the solitary sleep group compared to the partial and full co-sleep group. This model led to a significant better fit $(-2 \log likelihood = 721.5)$ compared to the model with only the time variables included $(-2 \log likeli)$ hood = 738.2), $\chi^2(3) = 16.7$, p < .001. Inclusion of the education variables (main effect and interactions with the time variables) did not lead to a better fit of the model (difference in $-2 \log$ likelihood: $\chi^2(3) = 4.5$, p = .20), and did not influence the effect of co-sleeping on cortisol reactivity. They were therefore not included in the final model. The regression results for the final model with the best fit are shown in Table 3.

These analyses indicate that the solitary sleep group has a different reactivity pattern over time than the partial and full co-sleep group. Fig. 2a shows the cortisol responses to the bathing session for the 3 groups. Post hoc one-way ANOVAs with co-sleeping as a betweensubject factor showed that there was a difference in cortisol levels at sample moments T2 (F(2, 109) = 4.79, p = .01) and T3 (F(2, 98) = 3.75, p < .05). There were no group differences at T1 (F(2, 112) = 1.19, p = .31). Post hoc t-tests on the contrast between the solitary sleep group versus the partial and full co-sleep groups (Solitary sleep dummy) showed that at moments T2 and T3 solitary sleepers showed significantly higher cortisol levels than partial and full co-sleepers (t(31) = 3.0, p < .05 and t(99) = 2.7, p < .01, respectively). Cortisol levels at moments T2 and T3 did not differ between the partial and full co-sleepers (ps > .56).

3.2.2. The vaccination

Next, we performed multilevel regression analyses on the cortisol reactivity to the vaccination. The confounding variables that differed between the co-sleep groups (i.e., maternal education and age, and breastfeeding) were added to the model, as well as their interactions with time and time squared (see Table 3, model with confounders). Maternal education and age had no significant effects on cortisol levels over time (all ps > .24). Breastfeeding in the first 2 months, and the interactions of breastfeeding with the time variables (linear and squared) were significant (B = 0.01, p = .05; B = -0.034, p = .02 and B = 0.016, p = .02, respectively, see Table 3), indicating a smaller cortisol reaction in breastfed infants compared to bottle-fed infants. Post hoc tests showed that infants that were breastfed more than others (based on a median split), showed higher cortisol levels before the vaccination (F(1, 122) = 4.36, p = .04), but not 25 or 40 min after the vaccination (ps > .19), indicating relatively lower corti-

	Bathing session				Vaccination session			
Factors	Model with confounders		Final model		Model with confounders		Final model	
	В	р	В	р	В	р	В	р
Intercept	3.24	.000	3.14	<.001	0.91	<.001	1.00	<.001
Time (linear)	2.40	.005	1.71	<.001	0.23	.55	0.30	<.001
Time squared	-1.11	.006	-0.70	<.001	-0.044	.81	-0.12	<.001
Breastfeeding	-0.13	.56	_	_	0.01	<.05	_	_
Time \times breastfeeding	0.28	.50	_	_	-0.034	<.05	_	_
Time squared \times breastfeeding	-0.009	.73	_	_	0.016	<.05	_	_
Education	0.02	.66	_	_	0.045	.24	_	_
Time \times education	-0.27	<.05	_	_	-0.028	.77	_	_
Time squared \times education	0.12	<.05	_	_	0.016	.72	_	_
Maternal age	_	_	_	_	0.00	.99	_	_
Time \times maternal age	_	_	_	_	0.0081	.47	_	_
Time squared \times maternal age	-	_	_	_	-0.0053	.33	_	_
Solitary sleep dummy	_	_	0.23	.14	_	_	_	_
Time $ imes$ solitary sleep dummy	_	_	-1.25	<.005	_	_	_	_
Time squared \times solitary sleep dummy	_	-	0.46	<.05	-	-	_	—

Table 3 Regression results from the longitudinal mixed-model analyses for the bathing session and the vaccination.

Note: Solitary sleep dummy = contrast of solitary sleepers (0) versus partial and full co-sleepers (1).

sol reactivity in relation to breastfeeding. We entered the breastfeeding variables into the next model.

We then added the two dummy variables that classified the three co-sleep groups, as well as the interactions between time, time squared and the two dummy variables. However, none of the co-sleep variables predicted cortisol reactivity (all ps > .18). Fig. 2b shows the cortisol responses to the vaccination for the 3 groups. Post hoc one-way ANOVAs with co-sleeping as a between-subject factor showed that there was indeed no significant difference between the three co-sleep groups at any of the time points, all ps > .18. Inclusion of the breastfeeding variables (main effect and interactions with the time variables) only led to a marginally better fit compared to the model with only the time variables ($\chi^2(3) = 7.75$, p = .051), and was therefore not included in the final regression model. The final regression model with the best fit is shown in Table 3, including only the time variables.

To predict cortisol reactivity to the vaccination in the previous analyses, co-sleeping was averaged over the first 7 weeks. To disentangle possible differential effects of co-sleeping in the first and second month on cortisol reactivity, we also entered co-sleeping in the first month (week 1-4) and second month (week 5-7) as separate predictors of cortisol reactivity in a mixed model. No different results were found. That is, neither co-sleeping in the first, nor second month independently predicted cortisol reactivity to the vaccination.

4. Discussion

This is the first study to prospectively investigate relations between parent—infant sleeping arrangements and cortisol reactivity in early infancy. We found that solitary sleepers, i.e. infants that slept 90% of the night (between 0000 h and 0800 h) or more in their own bedroom, respond with a higher cortisol reactivity to a mild (bathing) stressor than partial or full co-sleepers. Several possible confounders, including breastfeeding, a measure of maternal caregiving quality, infant night waking, and sleep duration, were taken into account in this study. Solitary sleepers received less breastfeeding and had mothers with lower education than cosleepers, and hence these variables were controlled for in the analyses. Breastfeeding did not significantly predict cortisol reactivity to either the bath or the vaccination. Lower education was associated with slightly higher cortisol levels after the bath, but solitary sleeping continued to predict unique variance in cortisol reactivity to the bathing session, next to maternal education.

No associations were found between co-sleeping in the first months and the cortisol response to a vaccination procedure. However, co-sleeping was associated with more breastfeeding in the first two months, and higher maternal age and education. Of these factors, breastfeeding was marginally associated with the cortisol response to the vaccination. Thus, solitary sleepers in the first months of life seem to respond with higher cortisol reactivity to a mild physical stressor, but their cortisol responses to a vaccination are similar to those of young infants that sleep in close proximity to their parents.

The results of the bathing session are in line with animal studies that report heightened reactivity to stressors after high levels of early parental separation (Aisa et al., 2007, 2008). As maternal caregiving, night waking and sleep duration were not related to co-sleeping, and as breastfeeding was not associated with cortisol reactivity to the bathing session, it may be that lower cortisol reactivity to this mild stressor in the co-sleep groups is related to more availability of the parents at night. More proximity to, and hence more or faster physical and vocal contact with the parents during the night may increase external stress regulation, leading to less

frequent high distress levels and hence to lowered stress sensitivity in other domains of life as well. Also, although we controlled for quality of maternal caregiving, as measured through sensitive and cooperative behavior during the bathing session, it is possible that the simple presence of the mother during a mild stressor, irrespective of caregiving quality, was enough to help regulate partial and full cosleeper's cortisol reactivity to the bathing session. These explanations do not exclude each other and could both partly explain the findings.

We found no relations between solitary sleeping and the cortisol response to a vaccination procedure, 3 weeks after the bathing session. As the vaccination is known to elicit relatively strong cortisol responses in general, it may be a less optimal stressor to show 'hyper-responsivity' in the HPA-axis in comparison to the bathing session (Keenan et al., 2007).

In the present study co-sleepers received more breastfeeding than solitary sleepers and more breastfeeding was marginally associated with higher basal cortisol levels at the time of the vaccination. Higher cortisol levels in breastfed infants have been reported before by <u>Cao et al. (2009)</u>. In the post-vaccination cortisol no effects of breastfeeding were found, indicating relatively lower cortisol reactivity in relation to breastfeeding. Higher basal levels may lead to less reactivity by itself (Law of Initial Value: Lacey, 1956; Wilder, 1957), although previous studies have indicated a link between breastfeeding and lower stress reactivity to painful stressors (Shah et al., 2007).

Interestingly, no differences were found between the cosleepers and solitary sleepers on our measure of maternal caregiving quality, or on the infants' sleep characteristics. We measured maternal caregiving quality with video observations of sensitivity and cooperation behaviors. These measures have been well validated before (e.g., <u>Albers et al., 2008;</u> Van Bakel and Riksen-Walraven, 2002), predict later mother-infant attachment relationships and infant functioning (e.g., Egeland et al., 1993; Van den Boom, 1994; Van Doesum et al., 2008), and are stable in the first two years (Kemppinen et al., 2006). However, we measured maternal caregiving quality during a relatively short period, in only one specific care situation. Possibly, extending the observation time and observing the dyad also in other caregiving situations might yield a more robust measure of maternal behavior that could be related to co-sleeping and/or cortisol reactivity.

With regard to the lack of differences in infants' sleep characteristics, while co-sleeping has before been associated with more night wakings (Cortesi et al., 2004; Mao et al., 2004), and has been found to affect sleep duration and quality (Hunsley and Thoman, 2002), Mao and colleagues found co-sleepers to have shorter wakings and hence similar sleep duration. This is in line with our findings of similar sleep durations in the different groups. The fact that we also found no differences in the number of night wakings could be due to our co-sleeping group consisting mainly of room-sharers, while the earlier findings on frequent night waking are based on bed-sharers. Sharing a bed with a caregiver could be linked to the infant waking more often, while sharing the room might not.

Finally, maternal age and education were higher in the cosleeping groups as compared to the solitary sleepers. While co-sleeping has in the past been related to lower social economic status (Weimer et al., 2002; Blair and Ball, 2004), this apparent contradiction may be due to culture. In the Netherlands, early independence of children is traditionally valued, and includes solitary sleeping arrangements from the start. It may be that older or more highly educated mothers are more willing to break traditions in the pursuit of their personal caregiving beliefs.

If the apparent heightened reactivity of the HPA-axis of solitary sleepers to (mild) stressors generalizes to later ages, it may explain the findings by Waynforth (2007) in 3- to 8year-old children. In Waynforth's study, children who had experienced *fewer* years of co-sleeping showed higher basal cortisol levels. This could hypothetically be due to sustained higher cortisol reactivity to mild stressors during infancy, leading to chronically elevated cortisol levels in childhood (Miller et al., 2007). Future studies will have to show whether early co-sleeping can also influence HPA-axis reactivity at later ages. In this regard, Lucas-Thompson et al. (2009) found solitary sleepers to show lowered cortisol reactivity to vaccinations at 6 and 12 months of age, while in the present study no differences in reactivity to a vaccination at 2 months were found between the groups. One possible explanation for this discrepancy in results is the difference in sleep data collection methodology: questionnaires covering the behavior of the last month versus daily diaries, and different quantifications of co-sleeping. Another is that co-sleeping at 6 and 12 months of age may have a different origin than cosleeping in the first two months (i.e. reactive versus planned; Keller and Goldberg, 2004), or that co-sleeping effects on cortisol reactivity are age-dependent (e.g. due to developing self-regulating abilities, which may change the need for parental proximity during the night). Clearly, the relations between early and later sleeping arrangements and HPA-axis regulatory mechanisms throughout the first year(s) of life are a relevant topic for future research.

Strong points of this study are that we collected daily sleep arrangements data for an extensive period in a relatively large group of young infants, and that cortisol reactivity to two different, effective early life stressors was assessed. Limitations are that although we measured many relevant confounders in the present study, we did not control for infant health, recent feedings, naps, or for recent stressors while these can all influence cortisol levels. Individual differences in maternal behavior during the vaccination were also not accounted for. In addition, because of the small group of bed sharers in our study, we defined co-sleeping as sleeping in the parents' bedroom without distinguishing between bed sharers and room sharers (Ball, 2003), while both groups may naturally differ in cortisol reactivity. Generalizability of these findings to other stressors and ages, and long term health outcomes remain open questions for future research. And as reasons to co-sleep differ per culture (Keller and Goldberg, 2004), these findings may not generalize outside of the Netherlands.

As we studied associations between sleeping arrangements and cortisol reactivity, we cannot draw any conclusions on causality. There may be underlying causes explaining both sleeping arrangements and cortisol reactivity, e.g., infant temperament or (distress) behavior. Also, as parents chose the sleeping arrangement for their infant, there may be differences between the nurturing elements of the environment for co-sleepers and solitary sleepers that we did not measure but may influence infants' stress reactivity. While we investigated several important candidates, including breastfeeding, maternal caregiving quality, and sleep characteristics (i.e. infant night wakings and sleep duration), future studies should investigate other potential confounders or underlying causes.

Based on these findings, we would suggest that co-sleeping in the first month of life may be beneficial, as it is associated with lowered infant cortisol responding to a mild daily physical stressor. The fact that in our study only a small part of the co-sleepers were actual bed-sharers suggests that mere proximity to the parents at night (by sleeping in a separate bed in the parent's room) may be sufficient. This is important given that bed-sharing has been associated with an increased risk of Sudden Infant Death Syndrome (SIDS, for discussions see Goldberg and Keller, 2007a; Ball, 2009). When considering the potential beneficial effects of co-sleeping (or bed-sharing), multiple health effects should be taken into account (e.g. SIDS or heightened arousal during infant sleep: Hunsley and Thoman, 2002; Goldberg and Keller, 2007b; Ball, 2009), as well as relations to later behavioral and emotional outcomes (e.g. social independence: Keller and Goldberg, 2004).

In sum, solitary sleeping in early infancy is associated with heightened cortisol responding to a bathing, but not a vaccination session, as compared to co-sleeping. Breastfeeding, maternal caregiving quality, and sleep characteristics of the infant could not explain this association. The underlying mechanisms therefore still have to be unraveled.

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Conflict of interest

None declared.

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