

Should Neonates Sleep Alone?

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Background: Maternal-neonate separation (MNS) in mammals is a model for studying the effects of stress on the development and function of physiological systems. In contrast, for humans, MNS is a Western norm and standard medical practice. However, the physiological impact of this is unknown. The physiological stress-response is orchestrated by the autonomic nervous system and heart rate variability (HRV) is a means of quantifying autonomic nervous system activity. Heart rate variability is influenced by level of arousal, which can be accurately quantified during sleep. Sleep is also essential for optimal early brain development.

Methods: To investigate the impact of MNS in humans, we measured HRV in 16 2-day-old full-term neonates sleeping in skin-to-skin contact with their mothers and sleeping alone, for 1 hour in each place, before discharge from hospital. Infant behavior was observed continuously and manually recorded according to a validated scale. Cardiac interbeat intervals and continuous electrocardiogram were recorded using two independent devices. Heart rate variability (taken only from sleep states to control for level of arousal) was analyzed in the frequency domain using a wavelet method.

Results: Results show a 176% increase in autonomic activity and an 86% decrease in quiet sleep duration during MNS compared with skin-to-skin contact.

Conclusions: Maternal-neonate separation is associated with a dramatic increase in HRV power, possibly indicative of central anxious autonomic arousal. Maternal-neonate separation also had a profoundly negative impact on quiet sleep duration. Maternal separation may be a stressor the human neonate is not well-evolved to cope with and may not be benign.

Key Words: Heart rate variability, hidden regulators, maternal-neonate separation, skin-to-skin contact, sleep, stress

Early researchers attributed the infant protest-despair response to maternal separation to disruption of an attachment bond (1). Subsequent research traces the origins of this higher-order affective bond to earlier, simpler maternal-neonatal interactions where maternal presence and behavior constitute a suite of lower-order factors, each responsible for regulating neonatal development in precise and dissociable ways (2–6). Early maternal-neonate separation (MNS) therefore disrupts multiple regulatory systems with effects that depend upon the nature of each individual system (6). For example, keeping separated rat pups in a warm environment prevented the normally observed decline in motor activity (previously likened to despair) but had no effect on heart rate, which decreased by 40% as usual. Infusing milk into the pup's stomach did, however, maintain perfectly normal heart rates (1).

Maternal behavior also integrates prenatal building blocks such as hard-wired behaviors and conditioned preferences with postnatal affective learning to form intermediate attachment objects (1,6). For example, during birth, dams deposit amniotic fluid onto their nipple lines creating a familiar olfactory cue that initially guides pups to the nipples, which they instinctively grasp. Thereafter, nipple odors, dry suckling, and milk are potent (e.g., opioid-mediated) reinforcers that bond pups to nipples (6). Later, bonding to mother as a whole and eventually infant self-regulation are mediated by higher-order mental representations that grow out of first experiences mediated by simpler maternal-neonatal regulatory interactions and central neuroaffective pathways (1,6).

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Unless unveiled by careful research, early maternal-neonatal regulatory interactions remain hidden regulators (1) and recent research demonstrates that such interactions exert inordinate effects on neuroaffective outcomes much later in life. In particular, central corticotrophin release hormone (CRH) and other circuitry regulating hypothalamus-pituitary-adrenal (HPA) and sympathetic adrenomedullary stress-response systems in rodents is exquisitely sensitive to early adverse experience (3,7,8), such as daily episodes of brief (e.g., 15 minutes [9] or 3 hours [4]) MNS or individual differences in maternal licking and grooming behavior (9,10).

MNS in Primates

Dettling *et al.* (11) introduced an early adversity protocol wherein common marmoset monkeys experience daily 30- to 120-minute sessions of MNS from the 2nd to 28th day of life (total separation = 9 hours). Using this schedule, which approximates some human neonatal care (12), they found that MNS induced acute increases in urinary cortisol, epinephrine, and norepinephrine. After MNS, neonates spent more time in the suckling position, displayed less social play, and made more distress vocalizations than nonseparated control animals. Using the same common marmoset protocol, Pryce *et al.* (13) found increased urinary norepinephrine and increased systolic blood pressure in MNS infants 1 year later. Behavioral and affective disturbances were also observed after 1 year. At adolescence (48 weeks), Law *et al.* (14,15) found changes in expression of stress-related genes in the anterior cingulate cortex and in hippocampal genes involved in synapse plasticity and function, and Arabadzisz *et al.* (16) found reduced hippocampal glucocorticoid and mineralocorticoid receptor messenger RNA levels. Sabatini *et al.* (17) found reduced expression of a nitrous oxide metabolism gene (*GUCY1A3*) in the amygdala of 3-month-old rhesus macaque monkeys separated from their mothers when 1 week old. Social behavior correlated positively and self-comforting behavior correlated negatively with gene expression.

Manageable stress is a healthy part of development, providing a stress inoculation effect (5), but thus far, common marmoset studies report only deleterious long-term changes, such as

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exaggerated acute HPA responses (5,12), disturbed sleep architecture (12), and features such as anhedonia (13) and decreased reward associated with depression (18). This is in keeping with the fact that early separation is not ecologically valid in nonhuman primates (5).

Hidden Interactions in Humans

Pregnancy leads to endocrine priming of the brain and parturition triggers the expression of maternal and neonatal behaviors that are highly conserved across species (19). In humans, Widstrom *et al.* (20) and Winberg (21) describe how babies placed naked between their mother's breasts immediately after birth display stereotyped prefeeding behavior, comprising spontaneous sucking and rooting movements followed by crawling with their arms and pushing with their feet to locate the breast, attach to the nipple, and begin suckling within the first hour of life. In studies involving washing one nipple to remove chemical odor cues, 22 out of 30 babies selected the unwashed nipple (22). Further studies revealed that odors emitted from maternal Montgomery tubercles evoked pouting mouth movements and intensified respiratory and cardiac responses, even during sleep (23), and the smell of colostrum caused increased blood flow in secondary olfactory cortex but only in babies between 6 and 24 hours old (24).

Although it is known that maternal-neonate skin-to-skin contact (SSC) in the first hours of life significantly increases breastfeeding rates many months later (25), the broad implications of disrupting low-level hidden maternal-neonatal regulatory interactions in humans are unclear. Western culture routinely separates mothers and neonates, and because of an association with sudden infant death syndrome (SIDS), the American Academy of Pediatrics advises against co-sleeping, recommending same room but different bed (26). Yet, mother-neonate co-sleeping with close physical contact is likely the sleep mode in which primate neonatal physiology evolved (27,28). Skin-to-skin contact is essentially opposite to MNS and ensures that even hidden regulatory interactions will proceed. The incidence of routine SSC after birth is increasing (25,29,30), including for preterm and low-birth-weight neonates (31–33). In one study, low-birth-weight, premature babies were randomly assigned to SSC or MNS (incubator) from birth. Six hours later, all SSC babies displayed optimal cardiorespiratory stability compared with less than half in MNS (34). Moreover, within this period, over 90 percent of MNS but fewer than 20 percent of SSC neonates developed cardiorespiratory or metabolic disturbances requiring prompt medical attention.

Preliminary evidence also associates SSC with improved neurodevelopmental outcomes (35). Feldman *et al.* (36,37) compared SSC for at least 1 hour a day for 2 weeks in 35 preterm infants and 35 non-SSC control subjects (average just over 2 hours SSC per day). At term, SSC infants had better organized sleep-wake cycling than control subjects plus longer periods of quiet sleep and shorter periods of active sleep, indicative of a more mature neurodevelopmental profile (38). At 6 months, SSC infants scored higher on the

Bayley Developmental Index and the Psychomotor Developmental Index (36). Premature babies held for at least 2 hours in SSC between feedings once daily for 8 weeks showed more mature sleep organization (39) and accelerated brain maturation after 8 weeks compared with control subjects (40). In animals, MNS from postnatal day 5 in guinea pigs (which are precocious and able to feed themselves) impairs neurogenesis, shortens radial glial cell processes, and impairs granule cell migration in the dentate gyrus at 6 to 7 weeks of age (41). Lastly, handling/separation (MNS) of rat pups for 15 or 180 minutes daily from birth resulted in fewer autonomic motor neurons in several key areas, including central amygdala, bed nucleus of stria terminalis, hypothalamus, and limbic cortex at 10 days old (9).

The benefits of SSC, together with the adverse impact of brief repeated MNS, in animals implicate MNS as a possible physiological stressor in humans (42,43). However, direct evidence that separation is stressful for human neonates is lacking. Heart rate variability (HRV) is a means of quantifying autonomic nervous system (ANS) activity (44,45). Because the ANS is integrally involved in orchestrating stress reactions, we reasoned HRV could be used to detect ANS changes as a proxy measure of stress. We, therefore, compared HRV in 2-day-old babies across SSC and MNS. To control for level of arousal, we restricted our analysis to periods of sleep because sleep levels can be reliably monitored.

Methods and Materials

Nineteen normal healthy full-term neonates (37+ weeks gestation) born by cesarean participated in a within-subject design. Mothers had no psychiatric/neurological history or physical complications and all babies had good Apgar scores (Table 1). Maternal-neonate dyads were a convenience sample by virtue of mothers staying 3 days in hospital postcesarean. Routine postnatal ward care for well neonates during sleep is loose swaddling in open bassinets next to mother's bed. Mothers gave written consent on postoperative day 1. The following day, after neonatal examination confirmed them fit for discharge and pending maternal discharge, three electrocardiogram (ECG) electrodes were applied to the neonate's chest and two to the back during breastfeeding. Neonates then spent an hour in SSC and an hour in MNS (2 hours total, order randomly counterbalanced by tossing a coin). During SSC, neonates were secured in a prone position on their mother's chest using a customized wrap-around shirt (46). During MNS, neonates were loosely swaddled in blankets according to ward routine and placed semiprone, left side down, facing toward mother in a bassinet next to her bed. Level of arousal was recorded every minute using the Anderson Behavioral State Scale (39), wherein state 1 equates with quiet sleep and states 2 to 4 encompass all the stages of active sleep. An uninterrupted series of interbeat intervals (IBI) was recorded from each neonate across MNS and SSC using an ambulatory monitoring system (chest electrodes; VU AMS, Vrije Universiteit, Amsterdam, The Netherlands). Continuous ECG was

Table 1. Clinical and Observational Data for the 16 Neonates Studied

	Maternal Age	Sex	Age Hours	Birth Mass	Apgar 1	Apgar 2	First Place	Prior SSC	QS SSC (sec)	QS MNS (sec)	AS SSC (sec)	AS MNS (sec)
Mean	27.5	10 males	53.0	3295.3	8.4	9.8	SSC 9	Yes 5	1199.6	325.7	2022.8	2292.8
^a	7.6	6 females	9.3	436.6	1.4	.4	MNS 7	No 11	159.7	51.1	201.0	256.7
<i>n</i>									14	7	16	16

Indications for cesarean were: fetal distress 5, cephalopelvic disproportion 2, previous cesarean 2, hypertension 4, other 3.

AS, active sleep; MNS, maternal-neonate separation; QS, quiet sleep; sec, seconds; SSC, skin-to-skin contact.

^aNumbers in this row without a descriptor are standard deviation of the mean or, for the last four columns, standard error of the mean.

simultaneously recorded using an Active II System (back electrodes; Biosemi, Amsterdam, The Netherlands). Each neonate's entire IBI series was visually inspected offline for artifact and every IBI was cross-checked against the continuous ECG record. Using this method, it was possible to obtain a complete IBI series for every neonate over the entire observation period.

High-frequency HRV power (HF) reflects vagal modulation of respiration (respiratory sinus arrhythmia) (45). Low-frequency HRV power (LF) reflects slightly longer cyclical changes approximately every 6 to 10 seconds linked predominantly to a sympathetic-parasympathetic baroreceptor reflex (47). Frequency domain wavelet analysis (48) of each neonate's entire IBI series performed in Matlab (MathWorks, Inc., Natick, Massachusetts) yielded raw HRV power values (ms^2/Hz) at .5-second intervals. Low-frequency (.04–.3 Hz) and HF (.3–1.3 Hz) power were extracted at each time point and each series was standardized by dividing by its standard deviation (49). Standardized LF and HF power for every .5-second interval spent in quiet sleep or active sleep were then separately averaged. Awake, feeding, and crying states were not analyzed. For comparison with another study (49), HRV power was expressed in normalized units. Time to enter quiet sleep (latency) was measured from the start of SSC or MNS to the onset of quiet sleep. Neonates who never entered quiet sleep were given latency values of 60 minutes. This study was approved by the University of Cape Town's Health Sciences Faculty Human Research Ethics Committee.

Statistical Analysis

Sixteen neonates (10 males) had sleep recordings in both places (2 remained fussy, 1 technical data loss). Because only 6 babies entered quiet sleep in both places (SSC and MNS), whereas all 16 babies entered active sleep in both places (Table 1), quiet sleep and active sleep are analyzed separately. Given the small sample size, nonparametric methods were applied to all quiet sleep data. A paired sample *t* test was applied to the (raw) active sleep duration data (which was normally distributed). Not all active sleep HRV power data had a normal distribution (Kolmogorov-Smirnov $D(16) < .05$ for HF SSC and LF MNS). Consequently, all active sleep HRV variables were \log_{10} -transformed (which rendered $D(16) > .16$ for all active sleep HRV variables). A 2×2 place (SSC, MNS) \times frequency (HF, LF) repeated-measures analysis of variance (ANOVA) was then performed. Statistical analysis was performed in SPSS (SPSS Inc., Chicago, Illinois) and exact two-tailed *p* values are reported.

Results

Mothers ranged in age from 17 to 40 years (mean 27 ± 7.6), mean gravidity 2.6, range 1 to 5. Five had undergone cesarean previously and in five cases fetal distress was part of the cesarean indication. All 5-minute Apgar scores were ≥ 9 and there were no postnatal complications. The mean birth weight of the babies was $3295 \pm 437\text{g}$ and their age when studied ranged from 42 to 74 hours (mean 53 ± 9.3). Subject details and sleep duration results across place are summarized in Table 1.

HRV

For quiet sleep, Wilcoxon signed rank tests revealed significantly higher LF power in MNS compared with SSC ($Z = -2.20, p = .031$), mean_{difference} \pm SE = $.625 \pm .185$. Mean HF power was also higher during MNS, but for this small sample ($n = 6$), this did not reach significance ($Z = -1.36, p = .219$), mean_{difference} \pm SE = $.684 \pm .442$ (Figure 1).

For active sleep, a 2×2 place (SSC, MNS) \times frequency (HF, LF) repeated-measures ANOVA on log-transformed HRV power data revealed main effects of both place [$F(15,1) = 19.204, p = .001$] and

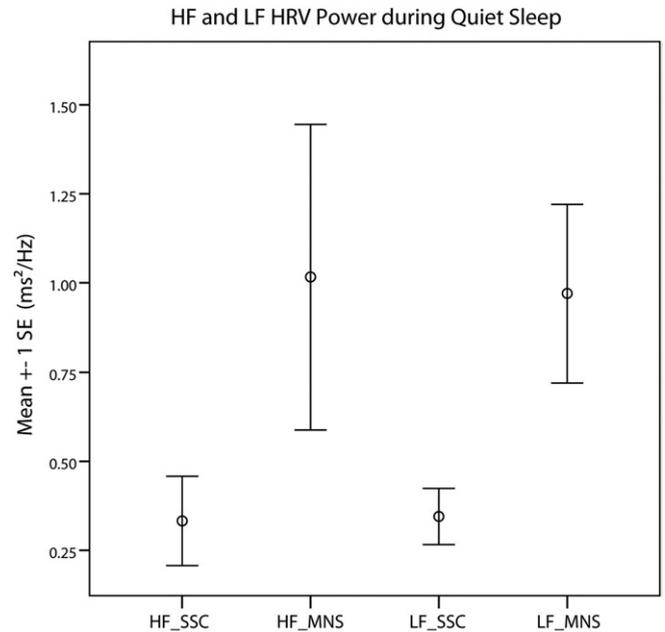


Figure 1. During quiet sleep, high-frequency (HF) heart rate variability (HRV) power was higher in maternal-neonate separation (MNS) than skin-to-skin contact (SSC), but this was not significant (mean_{difference} \pm SE = $.684 \pm .442, p = .219$). Low-frequency (LF) HRV power was significantly higher in MNS than SSC (mean_{difference} \pm SE = $.625 \pm .185, p = .031$). Given the large difference in means for HF, failure to reach statistical significance is almost certainly because of inadequate power ($n = 6$), i.e., whereas 14 out of 16 babies entered quiet sleep during SSC, only 6 of these babies entered quiet sleep during MNS.

frequency [$F(15,1) = 74.55, p < .001$] but no place \times frequency interaction [$F(15,1) = .153, p = .701$]. Paired samples *t* tests confirmed that HRV power was significantly higher in MNS than SSC for both HF [$t(15) = -4.12, p = .001$], mean_{difference} \pm SE = $1.028 \pm .254$, and LF [$t(15) = -4.38, p = .001$], mean_{difference} \pm SE = $1.787 \pm .485$ (Figure 2).

Because the frequency main effect likely reflects the $1/f$ (f = frequency) nature of the HRV power spectrum (50) and because LF and HF power behave in a similar manner across place, we report total HRV power (LF + HF) as a global index of ANS activity (Figure 3). Heart rate variability power across all sleep and frequency conditions was significantly higher in MNS than SSC [$t(15) = -4.721, p < .001$], mean_{MNS} \pm SE = 2.099 ± 1.187 , mean_{SSC} \pm SE = $.759 \pm .458$, a mean increase of 176% (Figure 3). Entering sex, fetal distress, and mass in the repeated-measures ANOVA yielded no significant effects (all $p > .14$).

Doyle *et al.* (49) recently published normative HRV data during quiet sleep and active sleep for a sample of 30 full-term neonates studied in MNS within 12 hours of birth. Our HRV results for quiet sleep and active sleep based solely on the six babies who entered quiet sleep during MNS closely resemble these findings (Figure 4). In both data sets, HF power expressed in normalized units shows a large significant decrease during active sleep ($Z = -2.028, p = .047$ for our sample). Low-frequency power expressed in normalized units increases in both data sets during active sleep, but this did not reach significance in our six babies.

Sleep

A Wilcoxon signed rank test revealed significantly longer quiet sleep duration in SSC compared with MNS ($Z = -3.24, p < .001$) (mean_{SSC} \pm SE = 1049 ± 638.8 seconds, mean_{MNS} \pm SE = $142 \pm$

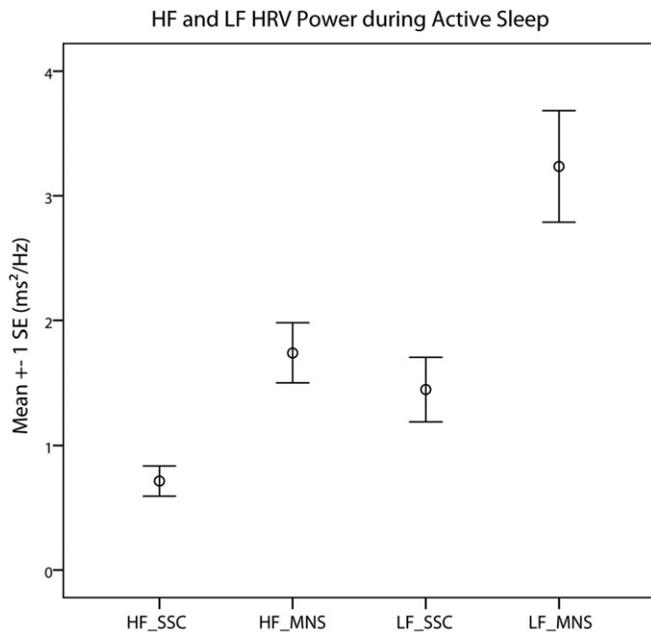


Figure 2. During active sleep, both high-frequency (mean_{difference} ± SE = 1.028 ± .254, $p = .001$) and low-frequency (mean_{difference} ± SE = 1.787 ± .485, $p = .001$) heart rate variability power were significantly higher in maternal-neonate separation than skin-to-skin contact. HF, high-frequency; HRV, heart rate variability; LF, low-frequency; MNS, maternal-neonate separation; SSC, skin-to-skin contact.

204.2 seconds), a mean decrease of 86% (Figure 5). The same test performed on only the six cases who entered quiet sleep in both SSC and MNS showed the same trend ($Z = -2.041$, $p = .063$), (mean_{SSC} ± SE = 1110 ± 118.7 seconds, mean_{MNS} ± SE = 330 ± 83.1 seconds). Although mean active sleep duration was longer in MNS than SSC, a paired sample t test revealed no significant difference [$t(15) = -.861$; $p = .403$], mean_{SSC} ± SE = 2022 ± 201.0 seconds, mean_{MNS} ± SE = 2292 ± 256.7 seconds) (Figure 5).

A paired sample t test also revealed significantly shorter latency to enter quiet sleep in SSC than MNS [$t(15) = -2.985$, $p = .009$], mean_{SSC} ± SE = 19.5 ± 3.7 minutes, mean_{MNS} ± SE = 38.1 ± 4.8 minutes (Figure 6).

Discussion

We compared HRV power while controlling for sleep state and duration in 2-day-old babies across SSC and MNS. Results show a 176% increase in combined high- and low-frequency HRV power indicative of robust autonomic activation (44,45) during sleep as a whole in MNS compared with SSC. Prior evidence comparing HRV across SSC and MNS is extremely limited and mostly confounded by a critical lack of control for level of arousal. Begum *et al.* (51) found significantly decreased LF and HF HRV power in 16 preterm neonates in SSC relative to MNS, and McCain *et al.* (52) found decreased HRV power, interpreted as decreasing stress, in a single preterm baby in SSC relative to MNS. In both cases, however, periods of quiet sleep and active sleep were not distinguished and quiet sleep duration was significantly increased in SSC relative to MNS. Because quiet sleep is associated with lower HRV power (53), it is impossible to ascribe the HRV changes seen in these studies to SSC per se. Nevertheless, despite the major methodological differences between these studies and ours, the results are broadly consistent, all pointing in the same direction of increased HRV power in MNS relative to SSC.

We also found an 86% decrease in quiet sleep duration in MNS compared with SSC. Although quiet sleep is associated with lower HRV power, decreased quiet sleep during MNS cannot explain the overall increase in HRV power during MNS relative to SSC, because in both SSC and MNS, there was much more active sleep than quiet sleep (Figure 5) and HRV power was much higher in active sleep than quiet sleep (Figures 1 and 2).

Temperature

The HRV power and quiet sleep changes we observed might result from bottom-up mechanisms as opposed to a top-down emotional response to separation (1,6). Cooling during MNS is a plausible bottom-up causal factor. Available data on the relationship between HRV power and temperature, however, suggests that cooling was not responsible for the HRV power differences observed. Cooling rats to a core temperature of 30°C elicited no change in HRV spectral power (54). In human neonates, a servocontrolled incubator study found maximal LF power at 35.5°C to 36.0°C. High-frequency power was maximal at 36°C and significantly lower at 35.5°C (55). From these results, a MNS cooling effect should decrease HRV power rather than increase it as we observed. Lastly,

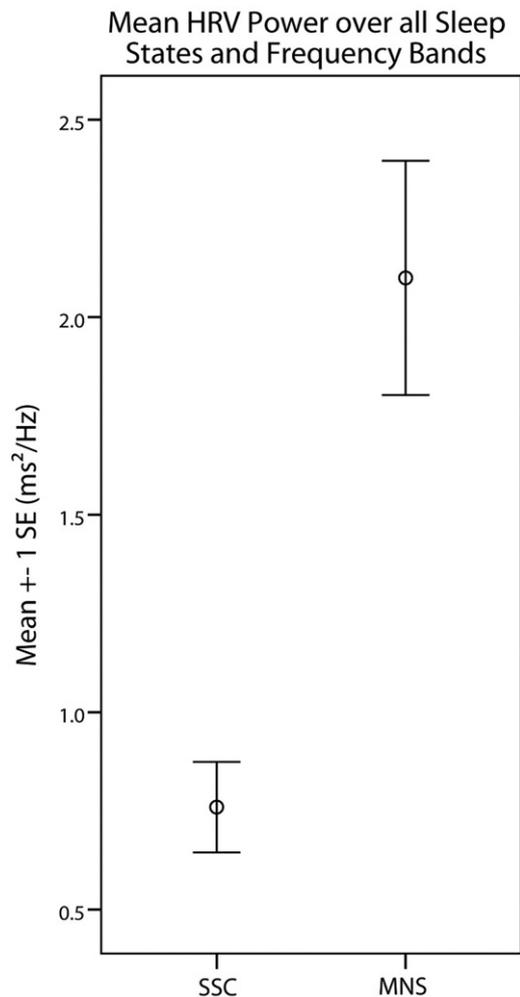


Figure 3. Heart rate variability (HRV) power across all sleep (quiet and active) and frequency (low-frequency plus high-frequency) conditions was significantly higher in maternal-neonate separation (MNS) (mean_{MNS} ± SE = 2.099 ± 1.187, $p < .001$) than skin-to-skin contact (SSC) (mean_{SSC} ± SE = .759 ± .458), a mean increase of 176%.

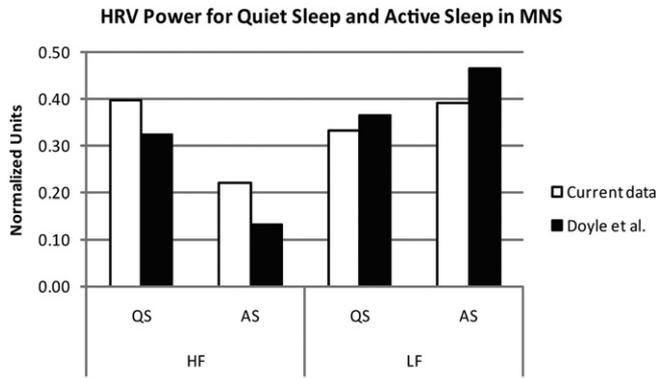


Figure 4. Comparison of mean heart rate variability power during maternal-neonate separation with that of Doyle *et al.* (49) for 30 neonates. Both datasets show the same pattern of changes across quiet sleep and active sleep. AS, active sleep; HF, high-frequency; HRV, heart rate variability; LF, low-frequency; MNS, maternal-neonate separation; QS, quiet sleep.

thermoregulation in adults is associated with very low frequency (<.05 Hz) HRV power increases as opposed to HF or LF subband changes (56,57).

Cooling is, however, known to decrease quiet sleep, because in human neonates, metabolic thermogenesis is initially more efficient at higher levels of arousal (58). In an incubator study, cooling by 2°C caused an increase in active sleep at the expense of quiet sleep, which decreased by 40% within 3 hours (58). There was, however, no further decrease in quiet sleep after 72 hours of sustained cooling, suggesting a lower limit to quiet sleep reduction in conditions of mild cooling. This reflects the fact that on cooling, neonates adopt a strategy of energy conservation as opposed to thermogenesis, i.e., metabolic rate ini-

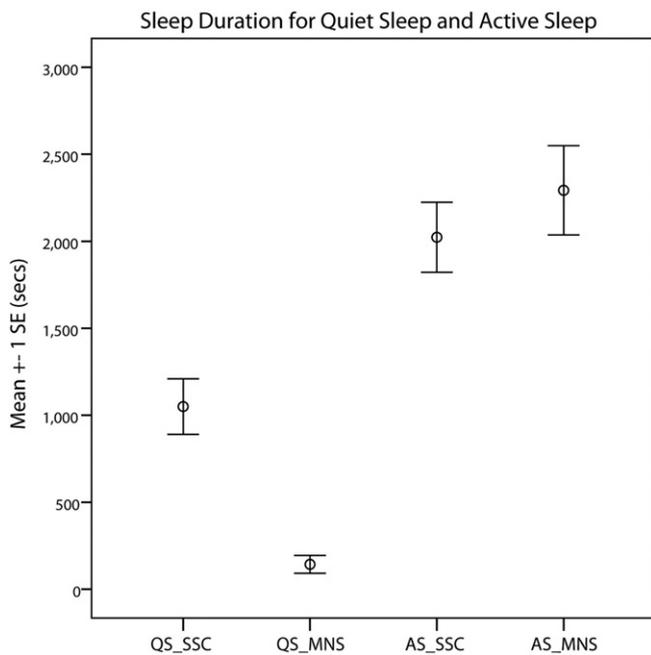


Figure 5. Sleep duration in skin-to-skin contact (SSC) and maternal-neonate separation (MNS). Quiet sleep (QS) was significantly longer in SSC (QS_SSC) than in MNS (QS_MNS) (mean_{SSC} ± SE = 1049 ± 638.8 seconds, mean_{MNS} ± SE = 142 ± 204.2 seconds, *p* < .001). Active sleep (AS) was not significantly different in SSC (AS_SSC) and MNS (AS_MNS) (mean_{SSC} ± SE = 2022 ± 201.0 seconds, mean_{MNS} ± SE = 2292 ± 256.7 seconds, *p* = .403).

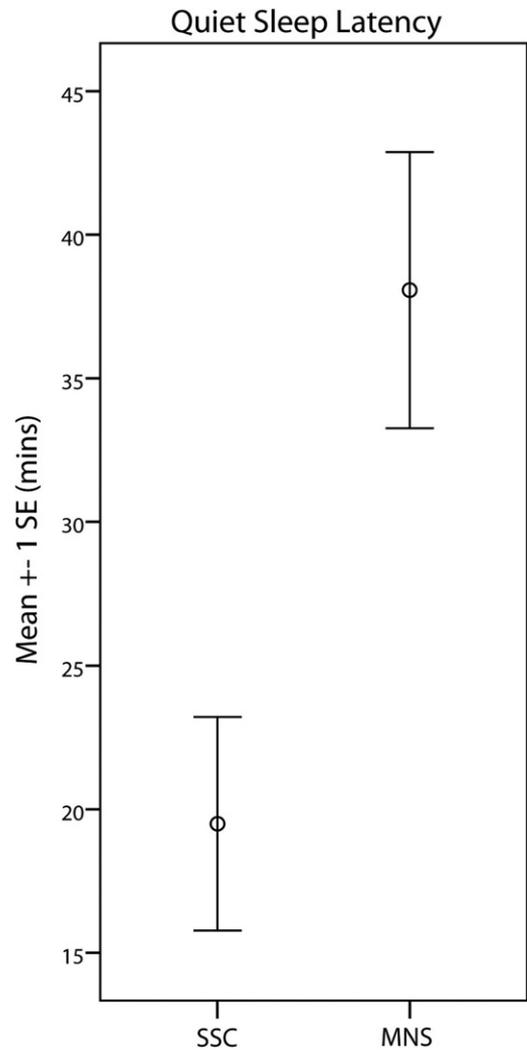


Figure 6. Quiet sleep latency was significantly shorter in skin-to-skin contact (SSC) than maternal-neonate separation (MNS) (mean_{SSC} ± SE = 19.5 ± 3.7 minutes, mean_{MNS} ± SE = 38.1 ± 4.8 minutes, *p* = .009).

tially decreases and core temperature is maintained within normal limits by reducing heat loss largely through peripheral vasoconstriction. After 72 hours of acclimation, thermogenesis rose above precooling levels and was now more efficient in quiet sleep than active sleep but still with no further change in percentage quiet sleep (58).

We did not measure infant temperature, as it is well established that SSC increases both peripheral and core temperatures in healthy newborns and preterm infants (25,59). Though statistically significant, core temperature increases in SSC are modest (< .5°C) and within normal clinical limits (25). Fransson *et al.* (60) measured abdominal, foot, and rectal temperature in SSC and MNS conditions similar to ours. Peripheral foot temperature decreased in MNS relative to SSC by 7.5°C, consistent with thermoregulation through peripheral vasoconstriction (58). It can be assumed that our babies responded to cooling during MNS in a similar manner.

There are, however, good reasons to not ascribe the quiet sleep decreases observed during MNS to thermoregulation alone. First, four human studies using incubators to control for temperature found approximately half the amount of quiet sleep in MNS com-

Table 2. Summary of Four Temperature-Controlled Studies Reporting Decreased Quiet Sleep During MNS Compared with SSC

Study	Time	Parameter Reported	SSC Value	MNS Value	Average Quiet Sleep Decrease (%)
Begum <i>et al.</i> (61)	MNS/Early SSC	Number of infants in quiet sleep at pre-specified time points	61.5	15.4	
	Late SSC/MNS		76.9	38.5	
Messmer <i>et al.</i> (63)	Average	Percentage time spent in quiet sleep	69.2	26.95	61
	MNS before SSC		25.55	13.6	47
	MNS after SSC			14.95	
Lal <i>et al.</i> (62)	Average	Number of 10-minute blocks spent in quiet sleep/hour	25.55	14.275	44
	Day 1		3.87	2.2	
	Day 2		4.13	1.8	
	Day 3		3.06	2.13	
Ludington-Hoe <i>et al.</i> (39)	Average	Not specified	3.69	2.04	45
	2- to 3-hour matched sessions SSC/MNS		Significantly less Quiet Sleep during MNS		
Current Results	1 hour each (SSC/MNS)	Average time (seconds) spent in quiet sleep	1049	142	86

MNS, maternal-neonate separation; SSC, skin-to-skin contact.

The percentage decrease in quiet sleep ranged between 44% and 61%. The current study found an 86% decrease in quiet sleep.

pared with SSC (39,61–63) (Table 2). Second, although thermoregulation-related changes in level of arousal during sleep in neonatal animals and humans differ markedly (64), animal studies that substituted the absence of maternal heat with an alternative heat source also report significant sleep disturbances. Hofer (65) reports abnormal sleep architecture in 2-week-old rat pups after 24 hours of maternal separation in a thermoregulated cage. Similarly, Reite *et al.* (66,67) consistently found disrupted sleep architecture in separated bonnet macaque infants who were immediately adopted by another adult female, with whom they slept as they would sleep with their mother. Barrett *et al.* (68) found that nursery- then peer-reared rhesus monkeys separated at 48 hours had altered activity and sleep patterns at 2 years of age, as well as a “fundamentally different relationship between waking cortisol and activity patterns” compared with maternal-reared monkeys. Third, unlike the babies in the incubator cooling study described above who increased active sleep at the expense of a 40% decrease in quiet sleep (58), our babies did not show a significant increase in active sleep during MNS despite an 86% decrease in quiet sleep. It therefore seems likely that a significant portion of the quiet sleep decrease observed in our babies during MNS is not attributable to thermoregulation.

Another factor against a purely bottom-up explanation of our findings is that the observed increase in HRV power during MNS is unlikely to be the cause of decreased quiet sleep in MNS. This is because increases in LF HRV power as large as 300% induced by artificial baroreflex activation appear to have no effect on sleep state, with babies remaining in quiet sleep for up to 10 minutes of such stimulation (53,69,70). These observations strongly suggest that increased HRV power per se is not responsible for the quiet sleep decrease observed in MNS.

Anxious Arousal

During MNS, bottom-up dysregulation of a hidden aspect of neonatal physiology can still activate central circuitry underlying anxious arousal (6), which, in turn, may result in top-down autonomic activation manifesting as increased HRV power. Because CRH is known to selectively interfere with quiet sleep, activation of central CRH-ergic stress-response circuitry in MNS (71,72) might

explain both the quiet sleep decreases and HRV power increases observed during MNS (73,74). Additionally, although cortisol feedback inhibits CRH, high cortisol levels acting directly on glucocorticoid receptors can also selectively interfere with quiet sleep (74). Thus, an MNS-induced central CRH-ergic stress-response activating both the ANS (indexed by increased HRV power) as well as the HPA axis in a top-down manner might have made it more difficult for babies to enter quiet sleep in MNS and when they did, made quiet sleep more difficult to maintain. In addition to decreased quiet sleep during MNS, the finding of longer sleep latencies in MNS supports the idea that babies also found it harder to enter quiet sleep during MNS (Figure 6). Lastly, HRV power during MNS was broadly increased across LF and HF also, perhaps suggesting top-down ANS activation rather than activity increases in more frequency-specific autonomic end organs (56,57).

Although the maternal factor(s) whose absence is responsible for the HRV increase and at least part of the quiet sleep decrease seen during MNS remain unknown, SSC inhibits crying immediately after birth (75). Maternal tactile-thermal stimulation during SSC may therefore be a hidden regulator inhibiting (or soothing) anxious arousal, possibly by downregulating a CRH-ergic stress response (71,72,76). The fact that brief daily SSC mitigates both against quiet sleep decreases outside of SSC (37,40; see also Table 2) and against longer-term adverse neurodevelopmental outcomes (35,37) suggests maternal tactile-thermal stimulation may be an important neuroprotective factor maintaining quiet sleep during SSC. The increase in quiet sleep after birth (64) is consistent with a neurodevelopmental role such as consolidating waking experience through repetitive synchronized activity in cortical-subcortical circuits (77). But even before birth, quiet sleep supports intrinsic maturational processes. For example, Milde *et al.* (78) recently demonstrated directional connectivity collapses between frontal and other cortical regions in normal full-term neonates. Collapses occur immediately before electroencephalogram interburst-burst events (trace’ alternant) and are widely thought to reflect neuronal reorganization processes underpinning the creation and strengthening of couplings between cortical, corticothalamic, and brainstem circuits responsible for burst generation (78–80). Because interburst-burst events only occur during quiet sleep, less quiet sleep during MNS

decreases the opportunity for cortical connectivity collapses indicative of functional reorganization within and between major cerebral structures. This could have far-reaching adverse neurodevelopmental consequences, especially in prematurity where trace' alternant is maximal.

Conclusion

The major finding of the present study was a striking increase in HRV power indicative of robust ANS activation (44,45) during sleep as a whole in MNS compared with SCC. Further studies controlling for temperature are necessary to see whether this difference is sustained during subsequent day-to-day episodes of MNS. Other physiological markers of acute stress (e.g., cortisol, catecholamines) should also be measured. In the interim, these preliminary results should be treated with caution. Nevertheless, given the clinical benefits of SSC (25) and the likely importance of quiet sleep in brain development (39), the finding in SSC of vastly increased quiet sleep and much lower ANS activity argues for SSC being the evolutionary expectation of the human neonate (81). In contrast, exaggerated ANS activity and minimal quiet sleep during MNS may reflect central stress circuitry activation with potentially harmful long-term neurodevelopmental ramifications. To the extent that SSC may prevent this, our observations support emerging trends in neonatal units, where parent-infant SSC is included as an integral element of proper care, even for the most premature infant, and separation avoided whenever possible (25,34).

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This article is dedicated to the memory of Judy Morgan.

Dr. Bergman disclosed he receives lecture fees for teaching and demonstrating on Skin-to-Skin Contact theory and techniques and produces promotional products for sale. Dr. Bergman and Dr. Morgan reported their participation in a patent application in the name of the University of Cape Town for a neonatal autonomic nervous system monitoring device. Dr. Horn reported no biomedical financial interests or potential conflicts of interest.

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